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16. ABSTRACT

This document provides conceptual guidelines for the development, implementation and evaluation of research task quality assurance plans for staff of the Health Effects Research Laboratory (HERL/RTP) of the U. S. Environmental Protection Agency, Research Triangle Park, North Carolina. It is designed to assist project officers in applying quality assurance concepts to each phase of a research task, from the initial planning through final report preparation. It is designed to assist the management staff in evaluating these plans and their implementation,for intramural as well as extramural tasks.

The guidelines describe the policy of HERL/RTP with respect to quality assurance, the structure of the quality assurance organization, and outlines specific quality assurance responsibilities for various staff positions. They also analyse the research task with respect to the various steps which project officers may take to ensure the highest possible data quality commensurate with resource limitations. Following this discussion, more specific guidelines relating to dosing activities and animal care are provided.

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GUIDES FOR QUALITY ASSURANCE IN ENVIRONMENTAL HEALTH RESEARCH

HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NORTH CAROLINA

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FOREWORD

The U. S. Environmental Protection Agency's Health Effects Research Laboratory located at Research Triangle Park, North Carolina conducts an extensive research program to evaluate the human health implications of environmental factors related to our industralized society. The purpose of this research is to provide information necessary to formulate environmental regulatory policies for the protection or improvement of public health and welfare while at the same time enhancing the nation's productivity. To this end, the Laboratory conducts a comprehensive environmental research program in toxicology, epidemiology, and research on human subjects under controlled laboratory conditions.

The quality of the data resulting from this research is an overriding factor in determining its usefulness in EPA's regulatory activities. In recognition of the importance of data quality assurance, our Laboratory has instituted an active, comprehensive program to coordinate the development and implementation of effective quality assurance planning into all research within the Laboratory. This document represents the current statement of our effort. I am confident that full implementation of our data quality assurance policy, with the help of the guideline manuals and the increased awareness of the importance of data validation and good management procedures, will enhance the scientific merit of our research program.

F. Gordon Hueter
Director
Health Effects Research Laboratory

ACKNOWLEDGEMENTS

These guidelines were completed through the auspicies of the Quality Assurance Committee, HERL-RTP. Committee members and other HERL research staff provided assistance and their valuable time to review, criticize, and make many helpful suggestions.

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CONTENTS

Page

1.0 SUMMARY.. 1-1
2.0 INTRODUCTION... 2-0
    2.1 Laboratory Mission............................... 2-1
    2.2 Data Quality at HERL/RTP......................... 2-1
    2.3 Definitions...................................... 2-2
        2.3.1 Quality.................................... 2-2
        2.3.2 Quality Assurance (QA)..................... 2-3
        2.3.3 Quality Control (QC)....................... 2-3
        2.3.4 Task....................................... 2-3
        2.3.5 Protocol................................... 2-4
3.0 MANAGEMENT POLICY.................................... 3-0
    3.1 Quality Goals.................................... 3-1
    3.2 Quality Policies................................. 3-2
        3.2.1 Task QA Design............................. 3-3
        3.2.2 Task QA Review and Approval................ 3-3
        3.2.3 Laboratory QA Program...................... 3-4
        3.2.4 Scope of Quality Assurance Program........ 3-4
    3.3 Quality Assurance Program Organization........... 3-5
        3.3.1 Organizational Structure for
              Quality Assurance 3-6
        3.3.2 Functional Responsibilities................ 3-8

4.0 GUIDELINES FOR PROJECT OFFICERS.................... 4-0
- 4.1 General.. 4-2
  - 4.1.1 Control Charts as Applied to Research Projects........................ 4-4
- 4.2 Experimental Design............................ 4-4
  - 4.2.1 Statistical Experimental Design.......... 4-5
  - 4.2.2 Quality Control Considerations........... 4-13
  - 4.2.3 Data Collection and Analysis............. 4-13
  - 4.2.4 Biological Systems....................... 4-15
- 4.3 Personnel...................................... 4-17
- 4.4 Facilities and Equipment....................... 4-19
- 4.5 Recordkeeping.................................. 4-22
- 4.6 Supplies....................................... 4-24
- 4.7 Sample Collection.............................. 4-27
- 4.8 Sample Analysis................................ 4-28
- 4.9 Internal Audits................................ 4-29
- 4.10 Preventive Maintenance........................ 4-31
- 4.11 Calibration................................... 4-33
- 4.12 Documentation Control......................... 4-41
- 4.13 Configuration Control......................... 4-42
- 4.14 Data Validation............................... 4-43
- 4.15 Feedback and Corrective Action................ 4-44
- 4.16 Data Processing and Analysis.................. 4-45
- 4.17 Report Design................................. 4-47

5.0 DATA QUALITY ASSURANCE FOR RESEARCH PROJECTS......... 4-0
- 5.1 Quantitative Estimates of Data Quality......... 5-1
- 5.2 Qualitative Samples of Data Quality............ 5-2

6.0 GUIDELINES FOR ATMOSPHERE GENERATION AND MONITORING.. 6-0
- 6.1 Introduction................................... 6-1
- 6.2 Atmosphere Generation.......................... 6-1
  - 6.2.1 General Considerations................... 6-2
  - 6.2.2 Particulate or Aerosol Atmospheres....... 6-4

6.3 Sample Collection and Analysis.................. 6-6
  6.3.1 Introduction.............................. 6-6
  6.3.2 Sample Representativity.................. 6-7
  6.3.3 Physical Characterization of the Atmosphere and Sampling Equipment......... 6-8
  6.3.4 Sample Quantity.......................... 6-8
  6.3.5 Sample Handling and Storage.............. 6-9
  6.3.6 Recommendations for Sampling and Analysis. 6-10

7.0 ANIMAL DOSING... 7-0

7.1 Preparation.. 7-1
  7.1.1 Preparation of Animals................... 7-1
  7.1.2 Preparation of the Test Substance......... 7-2
  7.1.3 Preparation of the Control Substance...... 7-3
  7.1.4 Preparation of the Vehicle................ 7-4
  7.1.5 Mixing.................................... 7-5

7.2 Administration..................................... 7-5

References.. R-0

FIGURES

Page

Figure 1. Functional Management Structure HERL/RTP............ 3-7

Figure 2. HERL Quality Assurance Organization.................. 3-9

Figure 3. Example of Major Topics Addressed in a Research Task Protocol.. 4-6

Figure 4. Proposed Protocol Contents EPA...................... 4-7

Figure 5. Summary of EPA's Proposed GLP's for Health Effects.... 4-8

Figure 6. Typical Calibration Curve.......................... 4-40

Figure 7. Minimum Report Technical Content for EPA Health Effects Tests.............................. 4-48

TABLES

Table 1. Currently Available NBS-SRMs for Environmental Research and Control................................. 4-36

Table 2. Summary of Measurements Methods for Selected Pollutants.. 6-11

SECTION 1

SUMMARY

This document is the statement of the Quality Assurance (QA) policy at the Health Effects Research Laboratory of the U.S. Environmental Protection Agency at Research Triangle Park, North Carolina (HERL/RTP). It provides guidelines for functional managers as they implement agency policy and evaluate research protocols. It provides guidelines for project officers as they develop and execute protocols for intramural and extramural research tasks in support of the HERL mission.

Since the necessity of assuring data of adequate quality pervades the entire scope of the HERL research effort, the QA program is designed to be correspondingly pervasive, including quality control and quality assurance planning and activities. Each aspect of the research task is analyzed from the perspective of designing, evaluating, and executing a research protocol to ensure adequate data quality.

The project officer holds primary responsibility for selecting specific quality assurance techniques and developing an appropriate quality assurance program for each of his tasks. Functional management is responsible for including evaluation of these quality assurance programs in the regular review and approval process, in the planning stages as well as during execution. The quality assurance organization is structured to assist project officers and management with task-specific problems and to evaluate and document laboratory-wide issues of data quality.

In order to aid both project officers and management in research task quality assurance, the research task is analyzed in detail in Section 4 in terms of its various operational phases, from the planning and experimental design through data quality aspects of the final report.

Following this discussion are QA guidelines for pollutant exposure and dose monitoring.

Programs for research quality assurance must evolve, since the concept of a formalized QA program and budget is relatively new to health research and since the research to which the QA program is applied, by nature, changes and develops. For this reason, the QA guidelines for HERL are extended, updated, and issued on a regular basis.

2.0 INTRODUCTION.. 2-1
  2.1 Laboratory Mission.................................. 2-1
  2.2 Data Quality at HERL/RTP............................ 2-1
  2.3 Definitions... 2-2
    2.3.1 Quality... 2-2
    2.3.2 Quality Assurance (QA).......................... 2-3
    2.3.3 Quality Control (QC)............................ 2-3
    2.3.4 Task.. 2-3
    2.3.5 Protocol.. 2-4

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2.1 LABORATORY MISSION

The Health Effects Research Laboratory, Research Triangle Park, North Carolina (HERL/RTP) conducts animal and human studies under controlled conditions and performs additional studies on human populations in order to assess the hazard to human health of exposure to environmental pollutants. Laboratory scientists determine the effects of environmental pollutants both alone and in combination; pollutant types that are studied include air pollutants, pesticides, toxic substances, and nonionizing radiation. Controlled laboratory studies are devoted to determining effects of pollutants on normal biological function as measured by clinical, chemical, biochemical, physiologic, histopathologic, growth, reproduction, and other parameters. HERL/RTP develops, evaluates, and improves analytical chemical methods and biological screening techniques for direct and indirect measurement of exposure to environmental toxicants. It also serves as a resource for information on the health effects of environmental pollutants and coordinates health-related activities with international organizations.

2.2 DATA QUALITY AT HERL/RTP

HERL/RTP has long recognized the importance of quality control in its research activities. For example, pesticide QA programs have been supported for several years and have been of service to over 100 laboratories. In addition to the pesticide analytical procedures, several related QA manuals have been prepared under this program. Interlaboratory pesticide QA programs have also been maintained for several years.

However, quality control has generally been practiced on a project-by-project basis, with the preparation and implementation of quality control activities being the decision of individual project

[illegible]
for a formally comprehensive, laboratory-wide data quality program.

A formal HERL/RTP data quality program was initiated in May 1976 with the issuance of a "Quality Assurance Plan" by HERL/RTP. Subsequently, a Quality Assurance Coordinator was appointed as chairman of the Quality Assurance Committee for the express purpose of designing and implementing a Laboratory-wide QA program appropriate to the unique requirements of the HERL/RTP. Quality assurance guidelines have since been developed and published for management policy [1] and for research task planning [2]. While specific quality assurance guidelines have been developed for environmental pollutant measurements [3,4,5], lack of adequate and comprehensive guidelines for quality assurance in biological research has hampered the completion of an integrated quality assurance program at HERL/RTP.

2.3 DEFINITIONS

The American Society for Quality Control has carefully defined terms that apply to quality [6] and is currently revising these definitions to reflect current understanding of quality terminology. The Quality Assurance Handbook for Air Pollution Measurement Systems [3] provides similar definitions of quality terminology applicable to air pollution data collection systems. For the sake of clarity, several terms related specifically to health research data quality are defined as they are used in these guidelines.

2.3.1 Quality

> **Quality means the totality of characteristics of research data that bear on their ability to satisfy previously specified criteria.** For laboratory measurement systems, accuracy, precision, and representativeness are characteristics of major importance. Completeness is an additional characteristic appropriately applied to larger systems, such as air monitoring networks.

2.3.2 Quality Assurance (QA)

Quality Assurance means planned, systematic actions that are necessary to ensure that the specified quality criteria are achieved. Thus, quality assurance (QA) planning is necessary at the management level in the development of QA policy. QA planning is also necessary in the development of the details of task protocols by project officers (see Section 2.3.5). QA activities result from QA planning and consist of a variety of activities. Quantitative measurements--such as calibration, interlaboratory tests, and analysis of "blind samples"-- are used. Qualitative measures--such as site visits by qualified professionals--are also used to evaluate the capability of a total measurement system for providing specified quality data. QA, in planning and execution, is a management function independent of task operating personnel.

2.3.3 Quality Control (QC)

Quality Control is a system of activities designed to achieve and maintain a previously specified level of quality in data collection, processing, and reporting. QC is performed by the organization actually carrying out the task or project; i.e., it is executed by task personnel. QC activities include control or correction for all variables suspected of affecting data quality. These variables are discussed in Section 4.

2.3.4 Task

A task is an in-house or contracted project or grant, or an interagency agreement, the purpose of which is to reproduce technical research data for the HERL/RTP program.

[illegible] be understood to include all task or project planning documents used at the [illegible]. Specifically included are research protocols, support activity procedure statements, contractors' work plans, and scopes-of-work, irrespective of the nature of the task or the organization actually performing the task.

Protocols are specifically understood to include plans for total task quality assurance--from the development of appropriate experimental design through the final report.

MANAGEMENT POLICY 3-1
3.1 Quality Goals 3-1
3.2 Quality Policies 3-2
    3.2.1 Task QA Design 3-3
    3.2.2 Task QA Review and Approval 3-3
    3.2.3 Laboratory QA Program 3-4
    3.2.4 Scope of Quality Assurance Program 3-4
3.3 Quality Assurance Program Organization 3-5
    3.3.1 Organizational Structure for Quality Assurance. 3-6
    3.3.2 Functional Responsibilities 3-8
        3.3.2.1 Program Coordinator 3-8
        3.3.2.2 Task Management 3-10
        3.3.2.3 Functional Management 3-11
        3.3.2.4 Quality Assurance Coordinator 3-12
        3.3.2.5 Quality Assurance Representative 3-13
        3.3.2.6 Quality Assurance Committee 3-13

The purpose of this section is to describe management policies and goals and the organizational structure for the implementation of a Quality Assurance program at HERL/RTP. The QA organization, consisting of a Quality Assurance Coordinator and a Quality Assurance Committee, serves in an advisory capacity to HERL/RTP professional and technical staff.

Planning for the application of QA measures is the responsibility of project officers. It is the responsibility of functional management to ensure that any project-oriented document or plan has incorporated appropriate QA measures. It is also the manager's responsibility to ensure that task QA plans are implemented and that task data quality is adequate for its intended purpose. To aid in carrying out these responsibilities, the QA organization is available to HERL technical and management personnel for consultation and, upon request, for active participation. EPA's commitment to QA is reflected in several ways (see, for example, reference [9]).

The goal of the HERL/RTP Quality Assurance Program is to ensure, assess, and document the medical and scientific reliability of laboratory and field data used in EPA's activities and documents relating to human health. Managerial, administrative, statistical, investigative, preventive, and corrective techniques are employed to maximize reliability of the data.

3.1 QUALITY GOALS

Specific goals of the HERL/RTP QA program are to:

a. Provide a vehicle, including an organizational structure, that will alert all personnel within HERL/RTP to

[illegible]

b. Establish and maintain [illegible] to support HERL/RTP personnel in the logical development of general and specific quality assurance plans for current and future HERL/RTP research.

c. Provide a means for evaluating proposed and ongoing tasks for appropriateness to the current and anticipated data requirements of HERL/RTP.

d. Implement, as part of the management plan, a procedure to review data quality aspects of research protocols and data currently being collected or data collected in the past, as deemed appropriate.

e. Encourage the use and development of methods of analysis and data treatment that are capable of meeting the data quality needs required by the HERL/RTP mission, as well as the use for which the data are intended.

f. Monitor the operational performance of HERL/RTP through appropriate intralaboratory and interlaboratory quality evaluation programs. This may be accomplished through cooperation with: evaluation services provided by other laboratories of ERC/RTP; other EPA laboratories; other governmental agencies (NIOSH, FDA, NBS); and private contractors.

g. Ensure that EPA project officers and contractors develop protocols with approved QA plans and procedures prior to task initiation and adhere to them in all stages of research.

h. Identify data quality problem areas and alert management to them. Also, validate the soundness of the solution to such problems.

3.2 QUALITY POLICIES

The HERL/RTP quality assurance program encompasses all funded technical tasks, intramural and extramural, contract and grant. Each research task protocol must contain a quality control plan delineating the QC practices and procedures to be followed at each level of task responsibility and each phase in the life of the project. Each

Responsibility for the design of a task QA plan rests with the respective project officer. As the professional most intimately familiar with the purpose(s) and procedures of the task, the project officer is the logical choice to assume this responsibility. He must choose specific QA activities appropriate to the data quality requirements of the task and to the specific nature of the data collection and data processing system. For instance, routine monitoring operations require QA plans and activities significantly different than those for measurement methods under development. Truly unique measurement methods are used quite rarely; well-characterized methods ("unit operations") are the norm, even for tasks that are highly research-oriented. Thus, the task QA plan focuses on specific unit operations and the data collection system in which they are employed.

QA plans for tasks conducted under grant or contract are prepared by the grantee or contractor and reviewed and approved by the EPA project officer (with optional assistance from the QA Coordinator). QA plans for in-house tasks are incorporated in the research protocol by the responsible project officer (again, with optional assistance from the QA Coordinator).

3.2.2 Task QA Review and Approval

Responsibility for the review and approval of task QA plans rests with HERL/RTP functional management. As an integral part of research planning, quality assurance plans (as they are implemented) provide the means by which functional management may assess that suitable data quality have been obtained in a cost-effective manner. Assistance from

The responsibility for the design and implementation of the Laboratory-wide QA program rests with the QA organization headed by the QA Coordinator.

The HERL/RTP Quality Assurance Coordinator, with assistance from the Quality Assurance Committee, establishes and administers quality assurance procedures for independently monitoring and assessing the adequacy of task quality assurance programs. The QA procedures should be applied uniformly throughout the duration of the project. However, at any time during the task life, either the respective project officer or the QA Coordinator, using accepted QA techniques, may assess the project's ongoing QA program as necessary.

3.2.4 Scope of Quality Assurance Program

The quality assurance program for extramural grants or tasks (contracts) provides for quality control procedures applied to the request for proposal (RFP), the proposal, and extending through proposal evaluation, work plan approval, project and quality control execution, and final report preparation. It also provides for appropriate data quality audits.

In the case of intramural tasks, quality control procedures begin with the drafting of the protocol). In particular, consideration of the hypothesis to be tested, data and data processing requirements, data quality assurance plans and procedures, data analysis techniques, and anticipated problem areas should be clearly addressed. Protocol review and approval includes evaluation of QA plans.

Planning for technical tasks should include provision for an appropriate QA program. This program will be comprised of both QC and

- Personnel
- Facilities and Equipment
- Recordkeeping
- Supplies
- Sample Collection
- Sample Analysis
- Internal Audits
- Preventive Maintenance
- Calibration
- Documentation Control
- Configuration Control
- Data Validation
- Feedback and Corrective Action
- Data Processing and Analysis
- Report Design

3.3 QUALITY ASSURANCE PROGRAM ORGANIZATION

In planning a QA program for a particular task, the project officer will attempt to account for all variables that are known or suspected to affect the data to be produced. Planning for such monitoring is not a simple task and performing it with the necessary care is still more difficult. However, it is becoming increasingly necessary to provide for such a QA program considering the number of reports which appear indicating that reagent quality and identity are not what the manufacturer claims them to be, instruments do not properly perform the function for which they are intended, electronic circuits are discovered to generate false signals due to mismatches, etc. Due to these general, and some specific, data quality problems, the EPA is currently developing comprehensive QA guidelines [7]. Federal standards for nonclinical laboratories have been promulgated [8] and health effects test standards have been proposed that will apply to testing under the Toxic Substances Control Act [9a,b]. The American Public Health Association published "Quality Assurance Practices in Health Laboratories," [10] and guidelines relating to health and biological systems, whether an isolated laboratory or involving an online monitoring system. The cost of this research is increasing at a corresponding rate. Efficient, reliable operation under such conditions requires systematically designed quality assurance plans for research tasks.

In order to better support HERL/RTP project officers and management in the rational design and execution of QA plans, the quality assurance substructure is interwoven within the existing management structure in HERL/RTP. The organization of this substructure, the functional responsibility of QA personnel, and the lines of communication for achievement of a cost-effective QA program are the subjects of this section.

3.3.1 Organizational Structure for Quality Assurance

The HERL/RTP functional management structure is shown in Figure 1. As noted above, one purpose of the Quality Assurance program is to encourage awareness and usage of quality assurance principles at all levels of HERL functional and task management. The Quality Assurance Coordinator, who reports on QA matters directly to the HERL/ RTP Laboratory Director, is primarily responsible for the design and implementation of the program. The Quality Assurance Committee, chaired by the QA Coordinator, is responsible for evaluating the effectiveness of the program throughout the laboratory and for recommending viable improvements. The QA Committee members act as liaison between the QA Committee and their respective Divisions or Offices.

In the standard review and approval process of any project-oriented document (e.g., RFP, proposal, work plan), the Quality Assurance plan (including provisions for QC and QA activities) is reviewed and approved along with the other technical or analytical aspects of the work. The actual definition and incorporation of

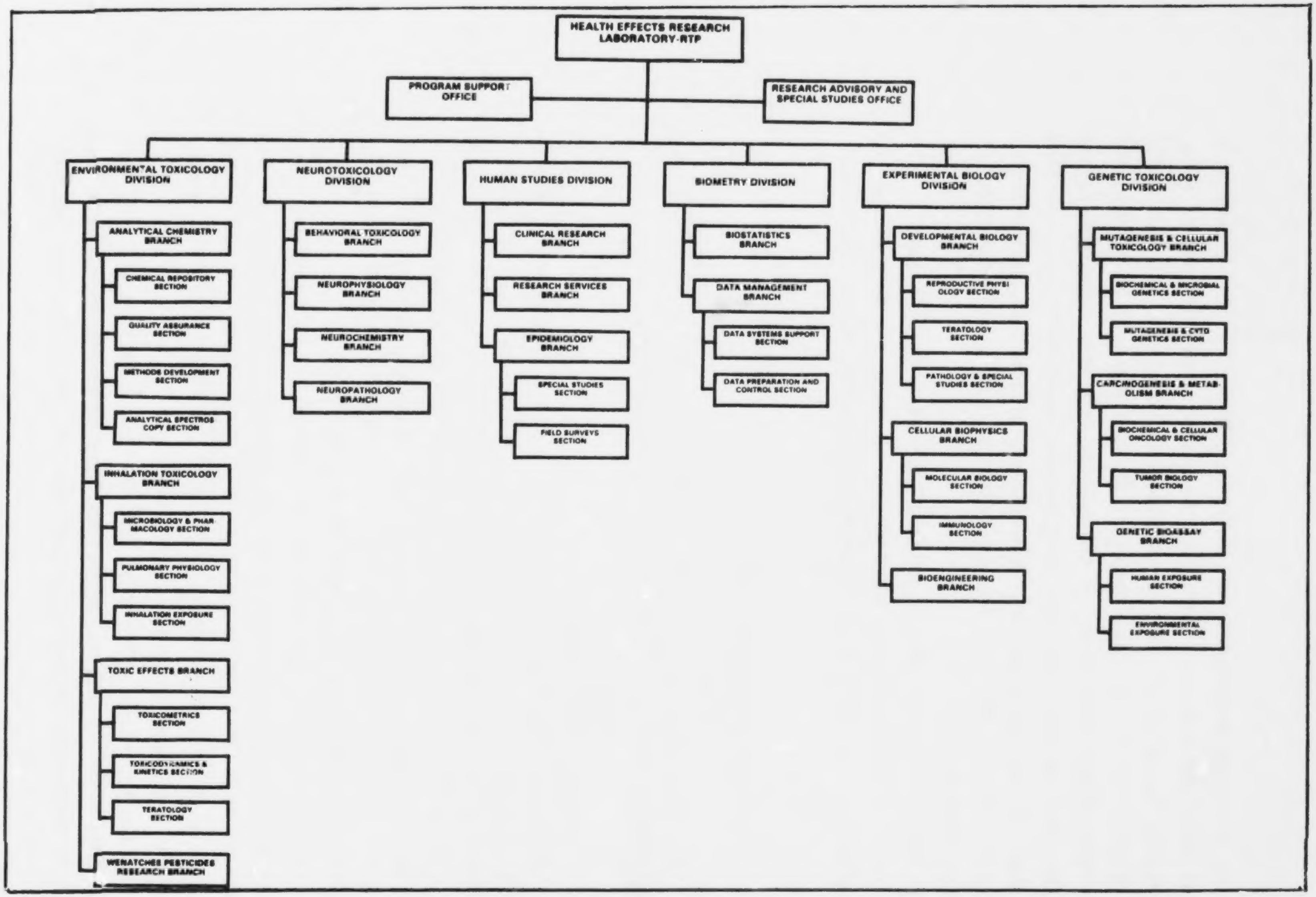
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Figure 1. Functional management structure, HERL/RTP

quality assurance procedures into individual tasks is the responsibility of each task's project officer. Beginning with the concept paper or request for proposal, all documents that contain a description of the technical or analytical aspects of the project must be accompanied by an appropriate description of the quality assurance requirements and how they will be met. The Quality Assurance Coordinator (or qualified designee) is available to work with project officer and management to design or refine the specific QA activities to meet laboratory QA program requirements.

Figure 2 depicts the QA organization as described above. The particular functions of each position are shown in the diagram and the channels of communication are delineated in the following subsections.

3.3.2 Functional Responsibilities

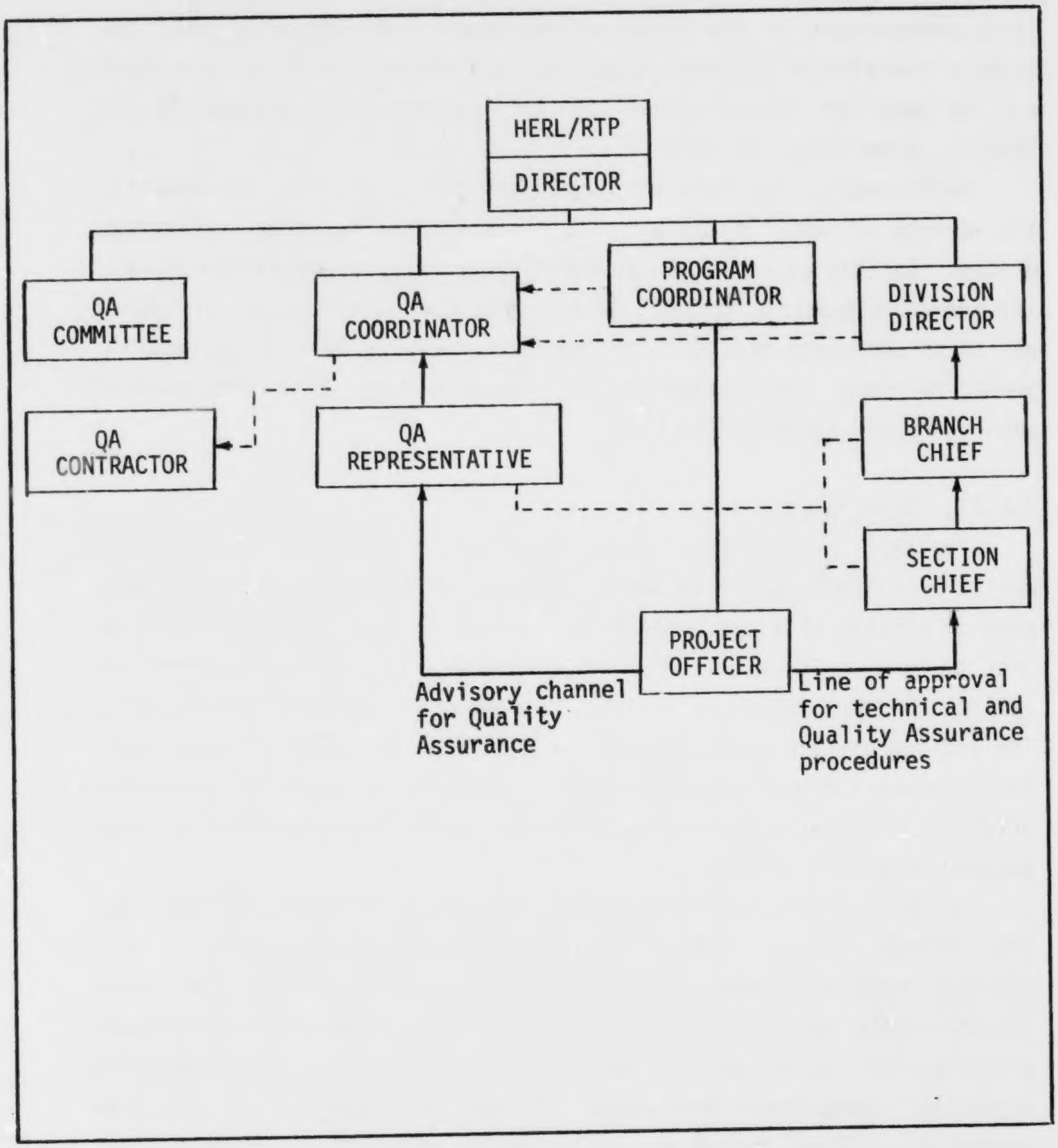
The functional responsibility assignments for individuals and organizational components are outlined in this section.

3.3.2.1 Program Coordinator---

Frequently, research quality assurance plans focus on technically related activities, such as calibration, acceptance testing, audits, and the like. The presumption is implicit that the data should be collected and attention focused on characterizing and controlling their quality. A thorough quality assurance program, however, includes provisions for specifically evaluating the desirability of collecting specific research data, apart from their quality.

In this context, the Program Coordinator is in an advantageous position to evaluate research proposals and protocols. By having ready access to interdisciplinary Decision Unit information, he can assess the relevance of each proposal (and task) to broadly defined Laboratory-wide and Agency-wide goals and needs.

As the reporting focal point for general program areas, the Program Coordinator is aware of correspondingly wide-ranging research data needs in these program areas. In addition to identifying and instigat-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DIRECTOR
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QA COORDINATOR
PROGRAM COORDINATOR
DIVISION DIRECTOR
QA CONTRACTOR
QA REPRESENTATIVE
BRANCH CHIEF
SECTION CHIEF
PROJECT OFFICER
Advisory channel for Quality Assurance
Line of approval for technical and Quality Assurance procedures

Figure 2. HERL quality assurance organization.

ing specific program-related research tasks, he evaluates related proposals and their relationship to other tasks in his program area. Thus, independent of the issue of the quality of research data, the Program Coordinator is responsible for evaluating issues such as cost and the need for obtaining one type of research data relative to the need for other types of data.

Additionally, the Program Coordinator is responsible for summarizing reports of work in his project areas for presentation to various groups. In this capacity he can identify research progress and impending needs in specific areas. He may thus word his reports in such a way as to encourage proposals for research tasks to fill these specific needs, aligning the production of research data with the general Laboratory and Agency needs.

3.3.2.2 Task Manager--

The HERL task manager (i.e., project officer) has the responsibility of fulfilling the technical and administrative requirements of a task or portion of a task. In order to fulfill this responsibility, he must be able to knowledgeably and adequately assure and document the quality of the task product; i.e., the research data and conclusions. The project officer draws upon his professional training and expertise, in collaboration with the HERL QA organization, to determine precisely which QA techniques most appropriately apply to a particular task quality assurance program.

In order to assure the technical aspects of research data quality, the project officer must plan ahead in systematic detail. This planning involves, among other things, anticipating events that might threaten data quality (e.g., slowly degrading reagents), contingency planning for unforeseeable failures and problems, and obtaining objective, independent evaluation of task data quality as the task progresses. These topics are sufficiently important to require their own discussion in Section 4 of this document.

The documentation of quality assurance activities is necessary in order to permit the communication and objective evaluation of task plans and results. HERL QA guidelines and activities are being developed in order to facilitate this requirement. In order to adequately document QA plans and activities, the project officer will need to become acquainted with technical and administrative aspects of HERL QA policy, explicitly include data quality considerations in the various task-related documents (e.g., RFP's, reports), and collaborate with the HERL QA organization in applicable QA activities (e.g., collaborative testing, split sampling--see Section 4.9)

3.3.2.3 Functional Manager--

With respect to the HERL QA program, functional managers are administratively responsible for ensuring the quality of research data that are produced under their direction. In this context, functional managers support the QA programs of project officers under their jurisdiction, as well as ensure that these QA programs are properly planned and implemented.

Management support of QA programs--for individual tasks as well as for Laboratory-wide effort--must be visible and active. Development and support of data evaluation techniques appropriate to health research data may be coordinated through the QA organization. Project officers using similar research techniques may be informed of, and encouraged to participate in, interlaboratory and intralaboratory testing programs. QA programs and techniques for many areas of health-oriented research may be readily developed using currently available standards and procedures. Functional managers should also actively support development of standards and QA procedures in new measurement areas. Coordination of effort by functional management in the development and application of effective QA techniques is essential to the development of the laboratory-wide QA program.

Management can ensure implementation of appropriate QA planning and techniques through the regular review and approval process. This can occur by requiring that all approved task documentation demonstrate concern for data quality by including a description of how data quality is evaluated and provided for in the ongoing task. Peer review of QA techniques, through the QA committee, may also be used effectively in the evaluation of task planning and execution. Section 4 of this document is designed to address critical aspects of data quality during the various evolutionary stages of a task. In this way, functional managers can use the contents of Section 4 to evaluate proposals, plans, progress reports, and final reports for their assessment of data quality.

These activities of functional managers serve to demonstrate to scientific and technical personnel the actual degree of commitment of HERL management to the quality assurance program.

3.3.2.4 Quality Assurance Coordinator--

The Quality Assurance Coordinator is responsible for the development, evaluation, and documentation of QA policy and procedures appropriate to the HERL mission. This includes evaluation of the cost effectiveness of QA programs and plans, and recommendations for their improvement. He also interacts with others involved in quality assurance programs through his professional contacts. As advisor to the Laboratory Director, he periodically reports on the progress and deficiencies of the Laboratory QA program and specific needs (e.g., method development and problem areas). He also recommends to the Laboratory Director specific courses of action for strengthening the HERL quality assurance program.

As chairman of the Quality Assurance Committee, the QA Coordinator initiates efforts to develop Laboratory-wide QA guidelines and procedures. He is the coordinator of methods development efforts for new QA procedures for specific HERL research techniques, and assimilates data provided by the Committee regarding evaluation of the QA program (e.g., weaknesses or the needs for new audit techniques). He is also responsible for the development of special audit programs for Laboratory-wide measurement techniques.

As quality assurance consultant, he is available to consult and recommend to the HERL professional staff (project officers, investigators, etc.) appropriate and necessary quality assurance methods and plans for ensuring the quality of the research data produced.

3.3.2.5 Quality Assurance Representative--

Each Division Director designates a QA representative, and an alternate, to serve as a member of the HERL QA Committee. The representative serves as a Division QA coordinator in that he consults on matters of quality assurance, serves as a source of information on research quality assurance matters, and is available to aid in implementing the QA program within his Division. As liaison with his Division, the representative is the prime source of information on QA matters.

As a committee member, the representative becomes increasingly aware of the requirements (and defects) of HERL QA policies and procedures with the aid of the QA Coordinator. He recommends and reviews proposals for improvements in QA policies and procedures. He also reports and evaluates (potential) data quality problem areas, as necessary.

3.3.2.6 Quality Assurance Committee--

The Quality Assurance Committee serves as an advisory committee to the Laboratory Director, with the objective of furthering the continuity and applicability of the Quality Assurance Program throughout HERL/RTP. Specifically, the committee's functions include assisting in the evaluation and refinement of data quality objectives of the QA program so that they meet the Laboratory needs with minimum disruption of existing workloads and procedures, reviewing recommendations presented to the committee, and assessing the effectiveness of the QA guidelines.

Page

4.0 GUIDELINES FOR PROJECT OFFICERS............................ 4-1
  4.1 General.. 4-2
    4.1.1 Control Charts as Applied to Research Projects.. 4-4
  4.2 Experimental Design.................................... 4-4
    4.2.1 Statistical Experimental Design................. 4-5
      4.2.1.1 Objectives and Hypotheses to be Tested. 4-9
      4.2.1.2 The Experimental Design................. 4-9
      4.2.1.3 Data Processing......................... 4-11
      4.2.1.4 Data Analysis........................... 4-12
    4.2.2 Quality Control Considerations.................. 4-13
    4.2.3 Data Collection and Analysis.................... 4-13
    4.2.4 Biological Systems.............................. 4-15
  4.3 Personnel.. 4-17
  4.4 Facilities and Equipment............................... 4-19
  4.5 Recordkeeping.. 4-22
  4.6 Supplies... 4-24
  4.7 Sample Collection...................................... 4-27
  4.8 Sample Analysis.. 4-28
  4.9 Internal Audits.. 4-29
  4.10 Preventive Maintenance................................ 4-31
  4.11 Calibration... 4-33
    4.11.1 Introduction................................... 4-33
    4.11.2 A Calibration Model............................ 4-35
      4.11.2.1 The Input Phase....................... 4-35
      4.11.2.2 The Operation Phase................... 4-38
      4.11.2.3 The Output Phase...................... 4-39
  4.12 Documentation Control................................. 4-41
  4.13 Configuration Control................................. 4-42
  4.14 Data Validation....................................... 4-43
  4.15 Feedback and Corrective Action........................ 4-44
  4.16 Data Processing and Analysis.......................... 4-45
  4.17 Report Design... 4-47

5.0 DATA QUALITY ASSURANCE FOR RESEARCH PROJECTS................ 5-1
  5.1 Quantitative Estimates of Data Quality................. 5-1
  5.2 Qualitative Estimates of Data Quality.................. 5-2

SECTION 4

GUIDELINES FOR PROJECT OFFICERS

The purpose of this section is to present guidelines for the development of quality assurance (QA) plans by project officers as they oversee the development and implementation of plans for individual intramural and extramural tasks at HERL/RTP. Specific purposes of this section are to:

a. Support the project officer in systematic planning for comprehensive quality assurance appropriate to all areas of his research.

b. Collect, in one section, general data quality checks.

c. Document data quality checks currently in use at HERL/RTP, for use by HERL/RTP professional and technical staff and other interested parties.

d. Provide a logical framework within which additional research relating to HERL/RTP data quality may be programmed.

The responsibility for the development and implementation of an appropriate QA plan for a research task rests with the respective project officer. Section 3.3.2.2 contains a general description of a project officer's responsibility for QA planning. This section includes specific discussion of quality control and quality assurance principles that may be used by all HERL/RTP project officers. The discussion of QA planning is organized to nominally parallel the sequence of events in the life of a research task. It is designed to be comprehensive and to complement the professional training and experience of HERL/RTP investigators.

Basic to the discussion which follows is the assumption that the research-trained project officer regularly performs various QA functions in his area of major expertise. These guidelines are intended to describe principles that complement and document these functions for every aspect of a research task that may be performed under the auspices of HERL/RTP; they do not provide solutions in detail. Research quality control (that is, activities to be performed by task operating personnel) is addressed in the following broad areas:

a. General approach to quality control in research.

b. Planning--experimental design, personnel, facilities and equipment, recordkeeping, supplies.

c. Experimental--sample collection, sample analysis.

d. Data quality activities--internal audits, preventive maintenance, calibration, documentation control, configuration control, data validation, feedback and corrective actions.

e. Results--data processing and analysis, report design.

Recommendations for quality assurance activities (i.e., independent of task operating personnel) are then discussed.

4.1 GENERAL

As performed at HERL/RTP, health-related research is frequently state-of-the-art, in concept as well as in technique. As such, it is not obviously susceptible to the normally available QA techniques. However, virtually every research or support task within HERL/RTP consists of two principal areas, whether the task is laboratory research, a monitoring program, or research support.

a. Data Collection and Processing--routine measurements performed by skilled technical personnel using well-characterized techniques (e.g., pH measurements).

b. Data Analysis and Reporting--nonroutine data analysis performed by the HERL investigator using physical models, statistical techniques, and other tools in a nonroutine, creative manner.

Each of these aspects of research is susceptible to the use of QA techniques by the project officer. Data collection techniques generally have adequately characterized quality control procedures associated with them that are quantitative in nature. The project officer uses professional judgment in determining the frequency, number, and specific reference materials to be used. Quality assurance of data analysis is less straightforward. Peer interaction, from the protocol stage to the report stage of a task, plays an important role. It is, therefore, important that effective mechanisms for peer review be used.

The production of research data is strongly affected by the "weak link" phenomenon. Thus, if experiment design, equipment maintenance, and data analysis are excellent and quality of the sample analysis is poor, the overall task data quality is lowered. Similarly, no amount of competent technical skills, data analysis, etc., can compensate for poor experimental design.

In addition, there are aspects of a research task which affect data quality, but which are not easily quantitated or categorized. For example, technician fatigue and morale should be considered. Similarly, the tension between the need for quick response to unexpected developments and the need for strict accountability to funding agencies relates to planning for quality data. With these considerations in mind, these guidelines are designed to support project officers as they oversee the progress of their tasks from concept to final report.

As a research project progresses, it frequently becomes apparent that additional "trivial" data (instrument settings, exact identity of the components of a buffer solution, etc.), which are not usually recorded, are useful for data interpretation. As a general rule, then, it is cost-effective to record well-organized, complete data from which an experiment can be properly reconstructed. Lab notebook (or station logbook) records of numerical as well as anecdotal data will frequently prove useful when experiment reconstruction becomes necessary.

The remainder of this section addresses the various elements of a research task. It should be realized that different research projects will involve varied applications of these QA elements. The project officer, however, should be cautious in deleting considerations of any element and should be certain that it will in no way affect the quality of the data that are produced by the task. If there is any uncertainty regarding the design of a task QA plan, the project officer may request the aid of his QA representative or the QA coordinator. It should be remembered that, when properly used, quality assurance planning can be a very effective insurance policy against data of unacceptably poor quality.

4.1.1 Control Charts as Applied to Research Projects

The theory and use of control charts as a tool for assuring and demonstrating data quality are described in Appendix H of reference 3. Use of this technique applies most often to routine, repetitious laboratory operations. Any repetitious operation can be documented on a control chart. Consider, for example, a task to investigate the effect of 6 months of exposure to a 2 parts per million (ppm) sulfur dioxide (SO_2) atmosphere on the respiration rates of white mice. Since the project may never have been done before and may never be repeated, it is not repetitious in itself and, therefore, cannot be subjected to control chart techniques. However, since the SO_2 atmosphere is to remain constant at a concentration of 2 ppm for 6 months, repeated determinations of that concentration are repetitious and can be subjected to control chart techniques.

4.2 EXPERIMENTAL DESIGN

Adequate planning prior to the startup of a task is by far the most cost-effective program for task quality assurance. This planning should include a discussion of the experimental design including manpower, facilities, supplies, and equipment logistics; and detailed plans for data collection and analysis as well as statistical experimental design per se. The protocol that results from this type of planning serves at least three purposes:

a. It provides a planning focal point for obtaining answers to the basic issues of:

   - -- what is to be accomplished?
   - -- how is it to be accomplished?
   - -- how can one show that the stated purpose has been accomplished?

b. It documents for all interested parties that responsible planning has occurred.

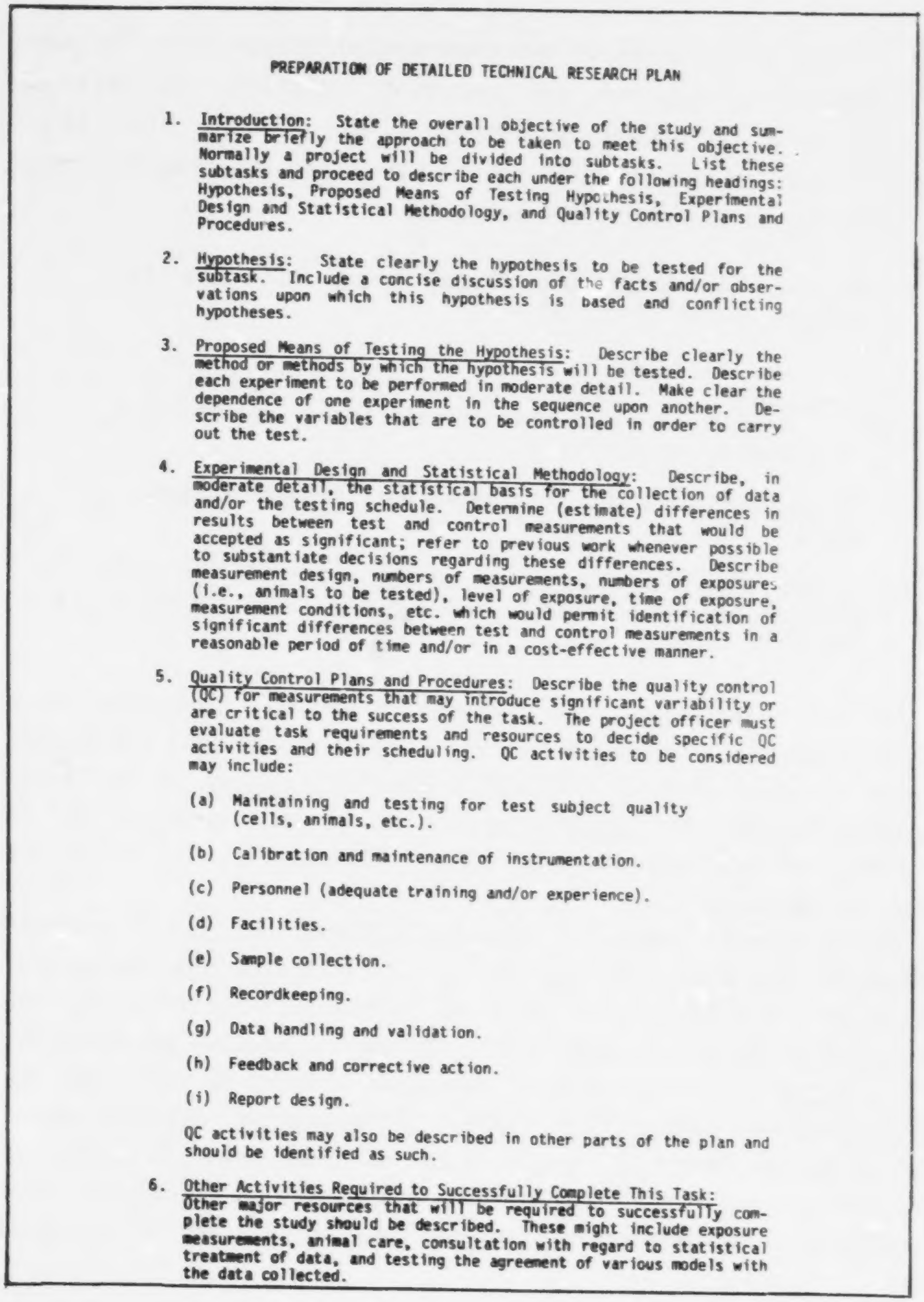
c. It provides criteria for making logical decisions when such decision points are reached in the later stages of the task life.

Typical contents of a task protocol (Figure 3), minimum contents of task protocol, as proposed by EPA as part of the Good Laboratory Practice Standards (GLP's) for Health Effects, relative to the Toxic Substances Control Act, [9] (Figure 4), may be used as guidelines in the design of task-specific protocols. Additionally, the GLP's are summarized in Figure 5.

During initial phases of research planning and during protocol development, the project officer should solicit advice from the various HERL support functions that will be involved. Specifically, the statistical design of the experiment, the data collection and analysis, and the animal care requirements should be planned in detail by the time the research protocol is drafted. (The ongoing collaboration of each of these functions should also be programmed in order to successfully cope with the various unexpected difficulties that generally occur in research.) Each of these three areas is discussed below.

4.2.1 Statistical Experimental Design

In any HERL task that involves the gathering and analysis of data, it is important to seek the aid of a competent statistician. The



PREPARATION OF DETAILED TECHNICAL RESEARCH PLAN

1. Introduction: State the overall objective of the study and summarize briefly the approach to be taken to meet this objective. Normally a project will be divided into subtasks. List these subtasks and proceed to describe each under the following headings: Hypothesis, Proposed Means of Testing Hypothesis, Experimental Design and Statistical Methodology, and Quality Control Plans and Procedures.

2. Hypothesis: State clearly the hypothesis to be tested for the subtask. Include a concise discussion of the facts and/or observations upon which this hypothesis is based and conflicting hypotheses.

3. Proposed Means of Testing the Hypothesis: Describe clearly the method or methods by which the hypothesis will be tested. Describe each experiment to be performed in moderate detail. Make clear the dependence of one experiment in the sequence upon another. Describe the variables that are to be controlled in order to carry out the test.

4. Experimental Design and Statistical Methodology: Describe, in moderate detail, the statistical basis for the collection of data and/or the testing schedule. Determine (estimate) differences in results between test and control measurements that would be accepted as significant; refer to previous work whenever possible to substantiate decisions regarding these differences. Describe measurement design, numbers of measurements, numbers of exposures (i.e., animals to be tested), level of exposure, time of exposure, measurement conditions, etc. which would permit identification of significant differences between test and control measurements in a reasonable period of time and/or in a cost-effective manner.

5. Quality Control Plans and Procedures: Describe the quality control (QC) for measurements that may introduce significant variability or are critical to the success of the task. The project officer must evaluate task requirements and resources to decide specific QC activities and their scheduling. QC activities to be considered may include:

   (a) Maintaining and testing for test subject quality (cells, animals, etc.).

   (b) Calibration and maintenance of instrumentation.

   (c) Personnel (adequate training and/or experience).

   (d) Facilities.

   (e) Sample collection.

   (f) Recordkeeping.

   (g) Data handling and validation.

   (h) Feedback and corrective action.

   (i) Report design.

   QC activities may also be described in other parts of the plan and should be identified as such.

6. Other Activities Required to Successfully Complete This Task: Other major resources that will be required to successfully complete the study should be described. These might include exposure measurements, animal care, consultation with regard to statistical treatment of data, and testing the agreement of various models with the data collected.

Figure 3. Example of major topics addressed in a research task protocol.

(A) A descriptive title and statement of the purpose of the study.

(B) Identification of the test and control substance by name, chemical abstract (CAS) number or code number.

(C) The name and address of the sponsor including the sponsor's project manager. The name and address of the testing facility at which the study is being conducted.

(D) The proposed starting and completion dates.

(E) Justification for selection of the test system.

(F) Where applicable, the number, body weight, range, sex, source of supply, species, strain, substrain, and age of the test system.

(G) The procdure for identification of the test system.

(H) A description of the study design, including the methods for control of bias.

(I) A description and/or identification of the diet used in the study as well as solvents, emulsifers and/or other materials used to solubilize or suspend the test or control substances before mixing with the carrier. The description must include specification for acceptable levels of contaminants that are reasonabley expected to be present in the dietary materials and are known to be capable of interfering with the purpose or conduct of the study if present at levels greater than established by the specifications.

(J) The route of administration and the reason for its choice.

(K) Each dosage level, expressed in milligrams per kilogram of body weight or other appropriate units, of the test or control substance to be administered and the method and frequency of administration.

(L) Method by which the degree of absorption of the test and control substances by the test system will be determined if necessary to achieve the objectives of the study.

(M) The type and frequency of tests, analyses, and measurements to be made.

(N) The records to be maintained.

(O) The date of approval of the protocol by the sponsor and the signature of the study director.

(P) A statement of the proposed statistical methods to be used.

Figure 4. Proposed protocol contents, EPA [9].

SUMMARY OF EPA's PROPOSED
GOOD LABORATORY PRACTICES FOR HEALTH EFFECTS
(FR Wed. May 9, 1979, p. 27369, ff)

a) The proposed GLP's apply to studies relating to health and safety evaluations conducted under Section 4 of the Toxic Substances Control Act, whether conducted by the sponsor, or under contract or grant. Fourteen terms are defined in this section.

b) **Test and control substances** must be characterized as to their strength, purity, composition and stability before the initiation of a study. Their **containers** must be labeled by name, chemical abstract number or code number, batch number, (expiration date) and storage conditions requirements. **Handling procedures** must be used which ensure proper identification, and minimize contamination, deterioration or damage. **Mixtures** must be suitably analysed to characterize their uniformity, concentration, and stability: expiration date is that of the earliest expiring component.

c) An adequate number of **personnel** having adequate and documented education, training, and/or experience must be available to the study. Their personal habits, health and clothing must be appropriate for their assigned duties. The designated **study director** ensures that all provisions of the GLP's are fulfilled for the study. The **quality assurance unit** independently ensures management that the facilities, equipment, personnel, methods, practices, records and controls are in conformance with the GLP's, in each phase of the study, at no more than 3 month intervals.

d) **Facilities** must be of suitable size, construction and location to facilitate proper conduct of the study. For animal studies, this means proper separation, isolation and quarantine of animals. Separate areas are required for: biohazardous substances; for diagnosis, treatment and control of known or suspected laboratory animal diseases; for sanitary disposal; for feed, bedding, supplies and equipment; for handling of test and control substances, and their mixing; for routine procedures; for administrative and personnel use; for secure archival of raw data and specimens.

e) **Equipment** must be suitably designed and located for operation, inspection, cleaning, maintanence and calibration according to written procedures; written records are kept to document these operations.

f) **Testing facility operation** must be by written standard operating procedures (SOP) for (as a minimum): animal room preparation; animal care; test and control substance handling; test system observations; lab tests; handling of moribund/dead animals; necropsy; specimen collection and identification; histopathology; data handling, storage and retreival; equipment maintanence and calibration; transfer, placement and identification of animals. All deviations must be authorized by the study director, and documented in the raw data. Each lab must have immediately available suitable lab manuals and SOP's, both active and historical. **Reagents and solutions** must be labeled to indicate identity, concentration, storage requirements and expiration date. SOP's for **animal care** include housing, feeding, handling, care, receiving quarantine, health parameters, identification. In addition, periodic feed and water analysis must be documented as part of the raw data; cages and racks must be cleaned at appropriate intervals. Bedding, cleaning materials and pest controls must be documented as noninterferring in the study.

g) Minimum **protocol** specifications are given (as in the HERL QA Guidelines document). The **conduct of the study** is detailed in terms of the protocol, specimen identity and records and data recording.

h) -i) Reserved.

j) Minimum contents of the final **report** are outlined (as in the HERL QA Guidelines document). **Archival** of all raw data, protocols, specimens and final reports is detailed: indexed, orderly and secure storage is required for at least 10 years.

k) **Inspection** of the testing facility must be permitted to an employee of EPA or FDA at reasonable times and manner: for records and specimens, not including QA records.

Figure 5. Summary of EPA's proposed
GLP's for health effects

statistician should be consulted not only after the data have been gathered but during the planning phase of the study as well. No analysis plan, however ingenious, can compensate for a bad experimental design. Subsequently, as the statistician is regularly involved in the daily execution of the plans, his timely advice for cost-effective midcourse changes will be a valuable asset to the maintenance of task data quality.

In general, the statistician's support throughout the task will be most helpful as the project officer formulates, examines, and carries out the following phases of the task:

a. The objectives and hypotheses to be tested.
b. The experimental design (i.e., the design of a testing program to meet the objectives).
c. The data processing plans.
d. The data analysis plan.

These four phases and the statistician's role in them are discussed below.

4.2.1.1 Objectives and Hypotheses to be Tested--

Determining the objectives and the hypotheses to be tested is obviously the first step that should be taken in designing any task. Precise written formulation of the questions to be answered enables one to state the hypotheses to be tested in precise terms and thus to plan a task more effectively. The aim should be to make the statement lucid and specific, avoiding vagueness or excessive ambition. It is advisable to classify objectives as major and minor. This classification is particularly helpful in assigning priorities to objectives when the task involves cooperation among people of different interests.

4.2.1.2 The Experimental Design (The Design of a Testing Program to Meet the Objectives)--

The testing program design should produce a clear definition of all the variables to be considered, the size of the testing program, the experimental units (e.g., animal models, cell cultures, humans) and exactly what data are to be collected. In designing the testing program, the following questions should be answered:

1. Are all the relevant factors (e.g., temperature and subject age) being considered?

2. Are the effects of the relevant variables adequately distinguishable from the effects of other variables (e.g., would a factorial design be more appropriate)?

   One can consider an experiment as intended to determine the effects of one or more variables (factors) on measures of experimental outcome. From substantive considerations, the project officer determines the factors, and the levels of each, that should be varied in his experimental program. In experiments involving two or more factors, the "effect" of a specified level of a particular factor may depend on the levels of other factors in the experiment (the factors may "interact"). The "main effect" of a factor is determined by comparisons among the effects of various levels of the factor. In designing multifactor experiments, the project officer should carefully consider what effects--main effect and interaction effects--are of interest to him. The experimental plan should be such that it will result in all the data necessary to estimate the main effects and interactions of interest at the end of the experiment.

3. Is the plan as free from bias as possible?

4. Does the plan use a historical measure of precision (experimental error) and if so is this precision sufficient to meet the objectives of the tests?

5. Is the scope of the testing plan consistent with the objectives given in Section 4.2.1.1?

6. Is the testing plan cost-effective (would a more limited test plan provide equivalent information at a lower cost)?

7. Are the data collection plans appropriate to the test objectives (are sample frequencies appropriate; should additional, or fewer, variables be monitored)?

8. Are available resources adequate for collecting the quality and quantity of data required?

9. Is the test plan logistically sound? (Is adequate time, space, manpower, etc., available to properly perform the quality checks necessary to ensure the specified data quality?)

Answering questions 1 through 9 allows the formulation of a scientifically sound, statistically suitable testing program and alternative testing designs. It is important to note here that the analysis of data (Section 4.1.1.4 below) can be made much easier if this phase (Section 4.2.1.2) is completed properly.

Finally, a complete description of the analysis scheme to be used in the task should be included in an experimental design. This scheme should include details of all pertinent parts of the task including sampling and data reduction as well as analysis. Advance development of such a scheme will aid in making decisions concerning other aspects of the task such as equipment or personnel qualification needs.

4.2.1.3 Data Processing--

The data processing phase of a task is concerned with how the data are handled once they have been collected, and involves examining the following kinds of questions about the data gathered according to the testing program formulated in the experimental design phase.

1. How are the data validated, i.e., what procedures are used to determine what data to include in the analysis? This question may involve developing a specific statistical evaluation of the data in a task and should usually be performed by the person or persons responsible for the analysis and interpretation of the data. Also, it should be clearly understood that experiments must not be repeated just because the results "don't look good." Section 4.2.3 contains further discussion of this point.

2. When are the data to be processed so that they can be analyzed, i.e., during the testing program or only at the end of data collection? This question is especially important if the test program extends over a long period of time, since preliminary analysis of the data

may indicate that the testing program should be altered for the remaining tests.

3. If data from different instruments are to be compared, what is the comparability of outputs (e.g., one instrument may give continuous readings while another may only give output at specific intervals)?

4. What (manual) data handling is required in order to convert "as recorded" raw data into the form in which they will be analyzed (e.g., copying from these forms and reading the cards into a computerized data base)? Also, what is a realistic estimate of the net error rate for this process (5 percent is a realistic value)?

4.2.1.4 Data Analysis--

Initially, this phase involves reviewing any data analysis that has been proposed or has already been performed on the project, and giving an outline of the analysis to be performed if no outline is available.

An outline of the data analysis should be prepared before the test design is completed or testing begins. If this outline is not prepared, it is quite likely that some measurements that should be recorded for proper analysis will be overlooked or will not be recorded in the correct manner. For example, an outline of the analysis may reveal that it is essential to record the level of an uncontrollable variable so that adjustments for the variable may be made when the data are analyzed. Conversely, unnecessary data may be identified and eliminated during this phase, thus conserving resources. In addition, if the project involves a large number of different types of measurements, it is important that an overall analysis plan be devised that insures that the objectives given in Section 4.2.1.1 are met in the most efficient manner. For example, a multivariate analysis may be preferable to several univariate analyses.

Once the data for the project have been gathered, the data analysis should be carried out with the close collaboration of a statistician. This is particularly important when the testing program has changed somewhat since the beginning of the project (which is frequently the case) and/or there is a large amount of missing data. In addition, the statistician and project officer should work closely together in presenting the results of the data analysis. In this regard the project officer should ensure that the presentation is understandable to nonstatisticians. The statistician should make sure that the results are presented such that the reader is aware of the functional relationship linking the data and the tables or graphs. The statistician should also ensure that statistical results are interpreted correctly based on the nature of the design and the statistical tests. Since any scientific study falls short of realism, useful conclusions usually require generalizations that tend to lie outside the realm of strict statistical justification. Thus, the reader of the technical report should be informed of the amount of statistical and physical justification supporting each conclusion.

4.2.2 Quality Control Considerations

Very early in the process of experimental design, consideration should be given to the methods that will be used to evaluate, control, and assure the quality of the experimental data. It cannot be overemphasized that this is one of the initial steps in the design process. EPA's commitment to QA is reflected in the directive "Environmental Protection Agency (EPA) Quality Assurance Policy Statement," a memo distributed May 30, 1979, in the publication of reference 9 and elsewhere. Far too often, quality assurance procedures are nonexistent simply because they were considered late in the project after all the money and time had been allocated. A design that can be expected to produce high quality data will have begun to incorporate effective quality control and quality assurance procedures at about the same time that the objectives were defined.

4.2.3 Data Collection and Analysis

Once the production of raw data has begun, the manner in which they are collected and analyzed becomes important. Data validation (see Section 4.14) must be addressed prior to this time. Manually collected data are frequently monitored by the person recording the data. However, computerized data acquisition systems do not have the potential for this treatment. They are known to pick up false voltage transients, and failure of one component of a system may seriously bias the data of major interest in an experiment. In a system of reasonable complexity, a variety of warnings may be identified by careful analysis of the relationships and patterns of values of the incoming data.

The use of control charts (see Section 4.1.1), or the concept, should be considered for use in specific data validation procedures. Used properly, individual out-of-range points and data trends will be readily apparent and informed response by the project officer will be possible.

The use of computerized data acquisition systems is increasing. This frequently permits a statistically acceptable, cost-effective extension of the control chart concept for real-time data validation. There are several advantages to using such a system. It accepts truly raw data to produce intermediate and final results in tabular or graphical form, thus minimizing human error. Similarly, the capability of rapidly and automatically comparing experimental data against recent values of similar data can serve as a real-time check on data validity.

Data analysis involves the matching of the experimental system with a model system and evaluating the differences. Since real-world data are never sampled exactly, one source of discrepancy between the data and the model is due to measurement error. Only rarely will the model exactly correspond to the test system, thus adding another component of data-model disagreements. The experiment should be designed so that data analysis will highlight the actual model-test system differences rather than mask the discrepancy as "error". Appropriate statistical design of the experiment is essential at this point. Care must also be taken that apparently irrelevant physical aspects of the test system do not produce data which lead to erroneous interpretations (e.g., diurnal fluctuations in serum enzyme levels are frequently larger than the response to the experimental stimuli on many biological systems). In order to maximize the quality of data from a testing program, the project officer should routinely consult with researchers who have specialized in related areas.

4.2.4 Biological Systems

The majority of the research and support associated with the HERL/RTP directly involves biological systems. While this is common knowledge among the HERL/RTP staff, it touches upon an important, and sometimes troublesome, difference between the experimental situation at the HERL/RTP and the situation at laboratories which perform research on nonbiological systems. The implication of this difference is that, while the experimental variables analyzed and modeled in other laboratories present a complex challenge, the experimental variables associated with biological systems studied at the HERL/RTP are orders of magnitude more complex. The "simple" systems under study in most physical science research laboratories involve the effects of a few to a few dozen experimental variables, most of which are monitored, if not controlled. Biological systems, even the most simple, involve the interactions among several dozen recognizable molecular species. And if research trends continue, several hundred distinctly recognizable molecular interactions will soon be characterized in the most simple monocellular systems.

The challenge of such a large array of experimental variables can presently best be met by permitting variation of only a selected few of these variables. For this reason the project officer must exercise his best professional abilities to recognize and fix all but the experimental variables. This is the purpose of care in selecting, maintaining, dosing, and analyzing biological subjects, whether they be cell cultures, animals, or humans.

Human subjects come from diverse and largely unknown backgrounds. This variability among human subjects can be minimized (but not eliminated) by careful pretest screening and questioning. The results thus obtained are directly applicable to human health problems. On the other hand, cell culture lines that have been quite thoroughly characterized for several generations are available for research. But the results of cell culture studies seldom, if ever, apply without interpretation to aspects of human health. Intermediate between these two extremes are animal subjects, some lines of which have been quite well characterized for several generations and which correlate closely with certain aspects of the human system. It is thus not surprising that a large proportion of health effects research is performed using animal subjects. Proper maintenance requirements of animals, however, are relatively more costly (in dollars and labor) than for cell cultures. Since careful characterization of animal subjects is no less important than for cell culture models, the balance of this subsection is devoted to a very brief discussion of animal care.

Comprehensive HERL/RTP guidelines for animal care are being developed, and general guidelines are presently available [12]. A brief discussion of the basic aspects of animal care is included here, due to its importance to overall task data quality. The basic concept, common to all scientific research, is to attempt to control all but the experimental variables. Early, intensive, and consistent consultation with qualified professionals from the HERL Laboratory Animal Support will maximize the quality of data that are generated using laboratory animals.

Animal selection should be based on awareness of the species' genetically determined immunities, as well as the specific dose-response relationship to be investigated. The research protocol should clearly state the basis for selection of a particular species, the anticipated interferences with the experiment design, and any preliminary testing required for adequate characterization of the system unknowns (e.g., interfering antibodies).

Acceptance testing, or prescreening and surveillance, should be sufficiently comprehensive to insure that only suitable animals are included as experimental subjects and controls. While the added expense of such testing may limit the quantity of animals used, the increase in data quality will generally more than compensate for this loss.

Personnel assigned to animal care and dosing should have sufficient technical competency to provide reliable routine care to experimental animals. In addition, their training and responsibilities should permit their active participation in the research (e.g., to note unusual behavior or health of any of the test animals or to note abnormalities in the dosing formulation).

The dosing and vehicle matrix should be chosen carefully and should be well characterized with respect to the specific experimental animals. If the particular choice has not been well characterized, it should be changed, or detailed studies performed to characterize it prior to experimental work. Choice of the control group and the specific regimen should be made on the basis of acceptable data quality, excepting only those aspects of the control that are reliably documented (i.e., complete equivalency of the experimental and control group regimen should be routine, excepting only the test substance).

In short, the animal subjects should be treated as any nonbiological supply; i.e., they should be thoroughly characterized.

4.3 PERSONNEL

Task operational personnel are intimately involved in one of the most crucial aspects of the particular research task: the generation and recording of the experimental cause-effect relationships that result in raw task data. The upper limit of the quality of the results is set during this phase of research task. Statistical treatment may be used to estimate precision and accuracy; creative thinking may rationalize discrepancies. But the upper limit of data quality for the task cannot be improved beyond what is produced by task personnel during task data collection. Two aspects of the personnel relationship to acceptable data quality are (a) technical qualifications and (b) the intangibles.

The usual approach to technical qualifications is that personnel have the education, training, and on-the-job experience to perform the assigned function. Similarly, training in good laboratory practice (generally and job-oriented) is recommended [8,9]. Such stipulations are certainly reasonable, and should be the documented practice of the project officer. Attempts should be made to ensure that all task personnel keep abreast of contemporary developments in their fields of expertise. Adequate theoretical briefing should be provided to bench technicians so that they will be capable of recognizing and recording unusual and unanticipated events.

In complex tasks it may be helpful to discuss personnel roles relative to the total task. By doing this, operating personnel can obtain a more complete perspective of their respective tasks, their interaction with others, and an overview of the experiment design. Periodic meetings during task implementation may help in information exchange, procedure standardization, and improved quality control of the project.

Another aspect relating personnel and data quality is far less tangible, but none-the-less important in obtaining high quality data. It refers to the general mental state of task personnel. Appropriate work loads prevent excessive mental and physical fatigue. Useless effort is avoided with optimum laboratory and equipment configurations. Good interpersonal relationships support full productivity. Proper management techniques (neither too restrictive nor to permissive) result in maximum productivity and data quality. In addition, the complex issue of motivation [13, Section 18] is an important factor in total personnel performance and data quality. The project officer is in the position to recognize and address such aspects relating to task personnel which create a healthy atmosphere for research and have a direct effect on overall task quality.

Bench-level personnel should also be intimately involved in the feedback and corrective action loop (Section 4.15). This involvement should begin early in the life of a task, preferably with a briefing on overall task goals, methods, and their role in assuring the necessary data quality.

4.4 FACILITIES AND EQUIPMENT

After the data required by a task have been identified, the requirements for facilities and equipment may be defined. The definition of these areas, like all areas of experimental design, should contain provisions for the assurance of data quality. The facilities and equipment selected for an investigation should be documented to be capable of producing acceptable quality data at minimum risk to task personnel (and subjects).

With HERL/RTP, the primary purpose of research conducted is to better model the responses of the human biological system. Frequently, nonhuman biological systems used for experimental purposes are selected with the intention of extrapolating results to characterize the human system. Due to the intentional similarity of the two systems, a significant risk of cross-contamination and infection is a constant threat to experimental results as well as personnel health. While it may be impractical or undesirable for the HERL/RTP investigator to strictly follow the various published animal facility guidelines, deviations should be made only at the advice and with the approval of the professional staff of HERL Laboratory Animal Staff (see Section 8).

Similarly, many nonbiological systems are used for health-related research, yet with potential risk to operating personnel. Insult to operating personnel by noxious fumes, electrical shock, etc., should be anticipated and eliminated as conducive to the long-range, cost-effective maintenance of data quality.

The experimental facility should be examine carefully prior to the commencement of experimentation. If it is a new facility, it will be most cost-effective to properly design the facility for its intended purposes. Modification of an existing facility is the usual case. In either case, resource (i.e., dollars, manpower, time, etc.) limitations always exist which directly and indirectly affect data quality. The various options, and their effects on data quality, should be frankly evaluated and discussed with the management. When the task involves a new experimental design in a facility already used by the investigators, de novo evaluation should be the norm. For a variety of reasons, this is difficult and may not be carried out. However, if a complete evaluation of the requirements of the experimental design, as well as of potential error sources, is conducted at the outset of a research project, future invalidation of much or all of the experimental work may be prevented. (For example, reference 14 reports that under certain conditions, light from fluorescent fixtures has caused mutations in the hamster cell chromosomes. If substantiated, these findings may bring into question an entire body of research. Rigorous attention to such seemingly trivial detail can minimize this type of problem.)

The need for dependability of support services should be evaluated early in considerations concerning facilities and equipment. Numerous measurement processes exist in which loss of routine services (such as gases, electricity, heat, steam, or water) causes significant deterioration in data accumulation or quality. In such cases it is necessary to provide redundant support services.

During the consideration of required conditions, it is necessary to examine detailed requirements for valid sampling. In air sampling systems the materials of which sampling lines, valves, and manifolds are constructed often play an important role in the condition of the sample when it reaches an analyzer. The geometry of the system also affects the validity of certain samples; the presence of long runs of tubing or bends and constrictions will change the character of certain types of samples (see Section 4.7). To assure high quality data, it is important to confirm that the monitoring system delivers to the analyzer a sample representative of the atmosphere being characterized.

In addition to the technical suitability of the facility for execution of the task, it is in the project officer's interest to evaluate and configure the facility with due care for the physical and mental comfort of the technical staff who will be using the facility. The discussion in Section 4.3 (Personnel) extends here to the human engineering of hoods (for poisonous and noxious gases), sinks, walkways, counters, etc. While there will be necessary trade-offs in facility configuration, the influence on traffic patterns, the environmental aspects such as temperature and lighting and other fatigue- and confusion-producing aspects should be evaluated and related to the effect on data quality.

Depending on the type of research involved, facility security should be specifically considered. This will range, for a wide variety of reasons, from areas available for common use by even nontask personnel to stringently restricted areas for safety purposes. Relating to data quality, the facility configuration should be carefully controlled (see Section 4.13, also reference 3, Section 1.4.19). As is frequently the case, even routine instrument maintenance activities can have a profound effect on data quality; for example, a new design of a replacement emission source for a spectrophotometer may affect data in a manner that only becomes apparent during later analysis. If possible, authority to approve facility configuration changes should be limited to one professional staff member who is qualified to document and evaluate such changes (i.e., the project officer).

As with the facility used for the task, the equipment should be evaluated for its applicability to the task research. The relationship of the measurement methods and the variables to be monitored should be well characterized during the initial task activities, if not before they have begun. Similarly, the subtleties of design and performance of different manufacturers' equipment should be thoroughly evaluated, preferably with the aid of a professional who has both theoretical and practical understanding of the specific instrument operation. In this regard, it is not uncommon to learn that unadvertised features of an instrument will permit acquisition of significantly higher quality and/or quantity data. As discussed below in relation to supplies, acceptance testing for new equipment should be performed on an item-by-item basis and documented for comparison with future testing. This testing program should be designed in such a way that operation of the instrument at its extreme limits (i.e., worst case), as well as routine settings, will be thoroughly characterized before it is made available for routine use.

In relation to equipment, the desirability of full- or part-time operator and/or maintenance support should be considered. Frequently, sophisticated instrumentation performs poorly or not at all when many occasional users have access to it. On the other hand, minor but frequent maintenance often keeps an instrument operating at peak performance. In such cases, the cost of a dedicated operator is justified.

4.5 RECORDKEEPING

Provision for a complete, permanent, easily accessible record of the raw experimental data should be made prior to, during, and following completion of task experimental work. This should include a written record (in ink, in a bound, page-numbered, durable notebook) of explicit identification of equipment, reagents, and supplies used, animal identification and test data, as well as a record of equipment and modifications and other seemingly inconsequential information which will permit more accurate analysis at later dates. A cross-referencing system must be used if the data are to be easily accessible following their initial use. Such a system may be of various levels of complexity, depending on the amount of data collected and their potential applications. Reference 8, Section J, lists rules for nonclinical laboratory reports and records, and their generation, storage, retrieval, and retention on a long-term basis. When data are logged by computers, it is important that adequate provisions be made for redundant and physically separate long-term storage of such records.

All technical personnel should be provided with a personal notebook in which they record all data, from weights and temperatures to calculations and general observations. Efforts should be made to encourage the entry of not only specific data (weights, absorbances, volumes, etc.), but also of anecdotal data (atmospheric or meteorological conditions, status of instruments, etc.), in ink. Erroneous or invalidated data should be indicated in such a way that the entry is flagged but remains legible. Drawing a single line through the entry is an acceptable indication, and this flag should be initialed. Whether the recorded data are valid or (flagged as) invalid, they may become extremely valuable in subsequent evaluation of a completed experiment or in initial planning of a related one. In general, the more data accumulated, properly recorded, and organized during an experiment, the more useful that experiment will be in satisfying overall task objectives.

At times it may be convenient to provide station, laboratory, or task data notebooks in addition to individual notebooks or project data notebooks. Such records will generally take the same form and adhere to the same recommendations as personal notebooks. The difference is that these books act as central records for the entire station, laboratory, or task, while the personal books act as records of individuals' contributions to tasks. Other means of recordkeeping include automated means such as strip charts, computer tapes, etc. Although these records are not of the same form as notebook records, the same recommendations apply.

Instrument (or equipment) log books contain all data relating to a particular piece of equipment. This log maintains a convenient record of instrument calibrations, maintenance, failures, and idiosyncrasies in one location. Reference to such a record provides an on-the-spot history of an instrument or piece of equipment that is often useful in determining trends, spare parts inventories, etc. Although equipment calibrations and maintenance records should be kept in such a log, a specific format or printed forms should be used for accumulation of such data. Such a format, when completed, will minimize the possibility of omission of important steps or data.

In addition to the issues discussed above, the project officer's investment in the design of suitable data logging forms for repetitively measured parameters will be repaid in the form of assurance of complete data, high productivity of technical personnel, and later, ease of reading the raw data. Computerized data acquisition systems have many advantages. However, they must be closely monitored for false or erroneous signals that may not be easily detectable.

High quality recordkeeping serves at least two useful functions: (a) it makes possible the detailed reanalysis of a set of data at a future time when the model has changed significantly, thus increasing the cost-effectiveness of the data; and (b) it may be used in support of the experimental conclusions if various aspects of the study are called into question. This latter point goes to the heart of scientific research: objectively, it is often possible to interpret data in more than one way and the raw data should be available for evaluation by qualified professionals; subjectively, when recordkeeping habits are sloppy, suspicion is quickly aroused that all other aspects of the research are of similarly poor quality.

4.6 SUPPLIES

As noted in Section 4.2.4 (Biological Systems), a basic premise of scientific research is that all but specified variables are controlled or held constant. However, reports regularly appear in the technical literature of impure and/or mislabeled supplies; e.g., after the end of expirements in which they were used, supposedly "germ-free" animal subjects are found to have been infected, thus invalidating the entire experiment. There are numerous examples available describing chemials, ordered to be 99.9 percent pure, which were found to have a 95 percent or, perhaps, even a 65 percent assay during acceptance screening [15].

An acceptance testing program for all incoming expendables/supplies--be they chemicals, biologicals, etc.--should be applied prior to and (judiciously) during use. Resources are always limited, hence the design of a suitable testing program is important. This is facilitated by learning as much of the processing history of the supplies as possible, by anticipating possible experimental interferences using the existing model, and by conferring with other users of the same consumable.

When a commodity is received as a supply for a task, it should be examined at once for acceptability. This acceptance screening will assure that supplies not meeting task specifications are not integrated into the task's supply stream. Acceptance screening for the HERL/RTP operations will deal with one of four classes of commodities: equipment, instrumentation, laboratory animals, or chemicals. The results of a successful test should (a) confirm the substance fully corresponds to the label specifications, and (b) confirm that known or suspected interferents are absent. When the acceptance testing is lengthy and/or costly, adequate amounts of a common lot should be purchased to permit completion of the tests. Sufficient excess to permit unanticipated testing, plus a specified amount for storage, should also be included.

Equipment and instrument screening should include all the testing required to demonstrate that the equipment or instrument performs according to the specifications under which it was ordered. Such testing, while protecting data quality, will also alleviate the problems, costs, and delays that will occur when it is shown that new equipment, already brought on line, is not performing to specifications.

The screening of laboratory animals presents a different problem altogether. Even though they may be regarded as a commodity, they are living and therefore susceptible to all the random variations applicable to living beings. Because of this complexity, quality assurance relating to laboratory animal care is the subject of Section 8.

The screening of chemical or reagent commodities should contain two elements--certification of assay and examination for impurities [16]. Such screening is usually performed on a batch basis. Certification of assay assures that chemicals arriving for use in a task are of the desired concentration or strength. In many analyses, chemicals having assays of considerably less than 100 percent may be utilized. However, the user of these chemicals must be aware of the decreased assay in order to make appropriate modifications in the computations.

An examination for impurities should be designed to assure that a chemical or reagent contains no substance(s) that may interfere with any analysis in which it is to be utilized. [A recent example of such an interference was discovered in EPA Reference Method 6 [17] for sulfur dioxide in stationary sources. This method utilized 2-propanol to separate interfering sulfur trioxide and sulfuric acid mist from sulfur dioxide. Certain lots of the alcohol have been found to contain oxidizing substances that prematurely remove the sulfur dioxide from the analysis screen. Thus, it is necessary to screen 2-propanol for oxidants prior to its use in Method 6.] Following successful completion of the acceptance test, an expiration date should be permanently marked on each container and it should be stored on a first-in-first-out basis. The shelf-life of many substances is known but in some cases it must be estimated. In most cases, simple tests exist that can, to a first approximation, rapidly document the strength and purity of a substance (or animal) immediately prior to use. Reliable estimates of strength as a function of time should be used to determine a conservative useable lifetime of solutions, mixtures, emulsions, etc.

In this latter instance, a well-designed central stockroom tracking system will facilitate rapid reference to the identity of other users of a substance. This will be useful for informal sharing of information of interest as well as for rapidly identifying and locating the users when a specific problem (e.g., purity or contamination) has been detected with the particular substance.

When chemicals must be stored for a length of time, certain conditions should be observed to protect the integrity of the material. These conditions will vary according to the specific chemical or piece of equipment and are best determined from the specifications of instructions for the material in question. However, parameters such as temperature, humidity, light, and shelf-life are usually of importance.

Since many of the substances involved in HERL/RTP are antagonistic toward humans, personnel should be protected from exposure to them. Certain substances are known to be in this category; however, the project officer should carefully evaluate whether additional substances may possibly degrade personnel health, and hence, data quality.

Special emphasis should be placed on the need to characterize all incoming cylinder gases containing pollutants in specified concentrations. The characterization should also include an identification of cylinder contents with reference to both pollutant(s) and matrix. It is well known that problems concerning the identity of cylinder contents and accuracy of the specified concentrations are commonplace. Even the best known and most reliable gas suppliers occasionally supply faulty materials. In addition, after the cylinder contents have been initially verified, experience indicates that over a period of time the contents degrade. Therefore, regular recertification must be performed to characterize changes in concentration, formation of new species, or loss of original species to prevent them from degrading task data quality. Because of these considerations, all HERL/RTP gas cylinders should be subjected to a rigorous program of initial, and regularly recurring, certification of contents and concentrations. Presently, the EPA Environmental Research Center is considering the establishment of a standards laboratory to serve this function.

4.7 SAMPLE COLLECTION

In sampling, one generates a new system, because as soon as a portion of material is removed from the whole, its history becomes different from the whole.* Primary consideration must be given to keeping the sample collection system as nearly representative of its condition when sampled as possible, regarding all the parameters under investigation. The processes involved in obtaining, holding, preserving, transporting, and resampling can potentially introduce significant direct and indirect changes in the material destined for analysis. Quality control measures must be specifically designed to quantitate and characterize any sample degradation or interaction with its particular container and environment.

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*A corollary to this is that the existing system is also altered by sampling activities.

Samples must be positively identifiable by those taking the sample and by others who are involved in subsequent analytical or handling steps. (This does not preclude the use of blind samples, spiked samples, or other audit methods to assure the quality of the test system in part or in whole.)

The personnel-related requirements for the technical and support aspects of the sample collection program vary in type and number. All operating personnel need to know exactly what is required of them, how it is to be done, and when it is to be done. Written instructions answering these questions for every phase of their involvement should be developed and provided as appropriate. Periodic "practice work" may be necessary in order to maintain the desired level of data quality. Each person should have a clear understanding of who will answer his questions on test protocol.

4.8 SAMPLE ANALYSIS

Sample analysis--whether it be spectrophotometer reading or viable colony count--involves a repeated sequence of similar, documented operations by technical personnel and/or automated instrumentation. For this reason, sample analysis is susceptible to the use of quality control techniques. Adequate, correct, and available operating procedures used by suitably trained and motivated technical personnel are the norm in a laboratory research context. Quality control activities on sample analysis range from nearly reflex use of a standard polymer film to calibrate an infrared spectrophotometer to the more visible use of split-sample aliquots, standard samples, and other techniques generally associated with calibration.

These latter activities require conscious and visible support and planning by the project officer if they are to succeed. Sample blanks should be analyzed on a regular basis. Samples spiked with known amounts of the analyte serve as a check on analytical bias. Split-sample aliquots can be analyzed by different analysts at different times using a different set of reagents as another measure of data quality.

Quality control measurements requiring highly developed subjective evaluations (e.g., pathological evaluation of tissue) may require side-by-side or round-robin analysis in order to establish the quality of the data. The project officer should choose the specific quality control activities appropriate to a given task in such a way as to emphasize the need for highest quality data commensurate with existing limitations.

4.9 INTERNAL AUDITS

During the life of a task it is desirable to regularly evaluate the ability of the total data system to produce data of the specified quality. In this way, timely corrective action (see Section 4.15) is possible. Internal audits, conducted by the operating group or organization, are used to obtain data for this evaluation.

The Environmental Protection Agency defines two types of audits which perform those functions [3,4]. A quantitative measure of the quality of the data produced is usually evaluated by means of a performance audit. The ability of a system to produce data of the specified quality is evaluated by means of a system audit; this type of audit is qualitative in nature.

The performance audit should be performed (a) by qualified task personnel not routinely involved in the measurement process, (b) in a manner that evaluates the data system in its totality. For example, an automated air monitoring system should be audited by introducing an appropriate known concentration gas into the sampling system inlet and recording the corresponding output from the data acquisition system. The same principles should be applied to laboratory instrument systems. Frequently the performance audit can only be designed to evaluate some discrete subsets of the total data system, such as sampling, analysis, and/or data reduction. Again, the audit should be designed and interpreted to evaluate each subsystem only to the extent possible within the context of the existing limitations. In either case, the audit values are compared with those generated by the data system(s), and conclusions are drawn as to the quality of the data being generated by the total system.

Tools available for use in performance audits generally fall into one of four categories:

a. Reference materials are available from several sources, most notably, the National Bureau of Standards [18,19], i.e., NBS-SRM's. These may be included for analysis in various types of measurement systems at relatively low cost with little interference to the normal laboratory routine and with the highest possible degree of confidence.

b. Reference devices may be obtained [e.g., the reference flow (ReF) device for high volume samplers] for which the critical parameters are known to the auditor but not the analyst. These are somewhat more disruptive of laboratory operations, and there is no possibility of anonymity of the sample; however, the final result is still a measure of the performance of the total analytical system, including the operator.

c. Cooperative analysis, such as round-robin analysis, is useful for estimating the precision (not accuracy unless the analyte is a reference material) of measurement among several different operators and/or laboratories.

d. Side-by-side analysis, or collaborative analysis, may be used if important variables are not controllable in the sample.

These basic types of audit techniques may be applied to almost any measurement system. Both EPA and NBS are expanding their services to allow calibration of many audit substances and devices for which no NBS-SRM's previously were available. Frequently, however, cooperative or side-by-side analysis will be necessary for internal audits of HERL analyses due to the lack of suitable reference materials or devices and the complex nature of the evaluation. In these cases, the project officer (or project leader for extramural tasks) will need to relate his responsibility to monitor and quantitatively document the task data quality with the various costs involved in this type of audit.

System audits are familiar to health-related researchers in the form of site visits by qualified professionals. Professional and technical evaluation, resulting from observation and discussion, is made of the capability of a data system (including instruments, personnel, organization, etc.) to produce the specified quality data. Questions such as:

-- Are there written sampling and analysis procedures and are they being used?

-- Are there written calibration procedures and are they used as frequently as necessary?

-- Is a preventive maintenance schedule defined and followed?

-- Are data reduction, validation, and reporting techniques completely documented and routinely utilized?

are answered on the basis of such observations.

It is extremely important to emphasize that the purpose of an audit is to constructively evaluate measurement process data quality (not personnel) and to identify areas where improvements can be made. If this intent is followed by project officers and managers and made clear from the beginning, personnel will be more likely to cooperate in audit and corrective action cycles.

In either situation, the program and rationale for internal audits should be designed on the basis of individual components of the specific measurement process and clearly planned for and budgeted into the task plans. By the use of internal audits, the project officer will be able to objectively evaluate data quality as his task progresses.

4.10 PREVENTIVE MAINTENANCE

In order to ensure long-term data quality in a cost-effective manner, a rational preventive maintenance (PM) program must be followed. This assumes importance roughly in proportion to the amount of instrumental data that are recorded. Reference 3 contains a good discussion of preventive maintenance, especially as related to routine measurements (Air Quality Monitoring). In particular, preventive maintenance will increase the completeness of data from continuous monitoring systems, which is an important measure of quality for such systems.

In a laboratory research environment, PM has a less visible benefit; the effect on minimizing and controlling equipment downtime is none-the-less real. Preventive maintenance can be budgeted and scheduled based on failure analysis data available to (or developed by) the equipment manufacturer. Extended laboratory use of specific items can be scheduled with higher reliability, and with shorter, less catastrophic interruptions than if maintenance only occurs following equipment failure.

The laboratory equipment PM program should include: scheduling, performance, and recordkeeping. Scheduling of PM should be developed based on the effect of equipment failure on data quality, any relevant site-specific effects, and equipment failure analysis (or estimates). This schedule should be available to the person or group responsible for performing the maintenance, as well as the person or group using the particular item of equipment. In this way, use of the equipment may be scheduled appropriately.

Preventive maintenance should be performed by qualified technicians, using accepted, documented procedures. The specific service should be programmed based on the considerations noted in the preceding paragraph and should be known to both the user and maintenance groups. A predefined set of data should be obtained both before and after the maintenance activities to permit equipment performance evaluation. Calibration (see Section 4.11) should also be performed following all maintenance activities.

Documentation of maintenance--scheduled or not--is essential to monitoring and documenting data quality. A bound notebook (see Section 4.5) should be kept with each instrument as a record of its maintenance history. A detailed description of adjustments made and parts replaced should be recorded in it. If the notebook is the multicopy type, one of the copies can be kept by the maintenance group for analysis. This analysis may include such considerations as mean time between failure (MTBF) for specific components, MTBF analysis for systems (individual and laboratory-wide), and indication of an onsite spare parts inventory appropriate to cost-effectively support minimum equipment downtime. Where possible, check-off forms should be used to ensure and document thorough maintenance activities.

4.11 CALIBRATION

4.11.1 Introduction

Calibration is the process of establishing the relationship of a measurement system output to a known input. In essence, calibration is the reproducible point to which all sample measurements can be correlated. This process is a key element of any scientific measurement program, since without an adequate calibration system, the validity of the data from the measurement program will be questionable.

A sound calibration system includes provisions for documentation of calibration procedure, frequency, conditions, and standards reflecting the calibration history of a particular measurement system.

Calibration should follow well-documented, step-by-step procedures to perform the needed referencing of a given system to a standard(s). Whether a specific standard is utilized for referencing, or visual analysis by trained personnel (e.g., pathologist reading a microscope slide), a clearly written, concise procedure will minimize the bias that may be introduced into a system due to individual differences. Calibration procedures for many systems can be obtained from NBS or ASTM. Other procedures may have to be developed in-house and must undergo extensive evaluation to determine, as nearly as possible, their accuracy, precision, replicability, repeatability, and reproducibility [3].

To assure and document that the calibration is being maintained for a measurement system, it is essential that calibration frequency be established on the basis of historically available data. As with preventive maintenance, the calibration frequency should be established on the basis of documented experience with specific equipment. Thus, initially, calibration frequency should be sufficiently high such that minimal drift is observed between successive calibrations. Only as this is done is it possible to rationally deduce a cost-effective frequency that minimizes exposure without jeopardizing data quality. The calibration schedule should involve simple daily checks as well as full-scale, multipoint calibrations. Provisions for action to be taken if an unforeseen circumstance occurs should be specified. Adherence to an exercise of this nature can minimize the generation of erroneous and/or indefensible data.

Environmental conditions are another type of reference point that must be dealt with during measurement systems calibration (and operation). If the system is sensitive to environmental conditions (temperature, pressure, light, humidity, etc.), the calibration will not be valid unless the documented conditions are maintained as required.

The quality of the calibration standards is the most important aspect of any calibration program; for without high quality standards, the accuracy of the calibration cannot be demonstrated. Standards should be of the highest possible quality and should be traceably referenced to a primary standard such as a National Bureau of Standards Standard Reference Material (NBS-SRM). Various organizations [18,19, 20] list reference materials applicable to health-related research for use by HERL/RTP project officers.

Documentation of each calibration, and the full history of all calibrations performed on a measurement system must be recorded. This enables personnel to perform a systematic review of the data quality from a measurement system at a later date.

4.11.2 A Calibration Model

In considering a general calibration scheme that can be applied to many different measurement processes, it is convenient to examine it as a model composed of three distinct phases. The input phase relates to preparation for calibration. It includes information on what standards and equipment are to be employed and the quality of the standards to be used. The operations phase relates to the steps and procedures by which calibration is to be accomplished. It includes such considerations as the detailed calibration procedure and operation of support equipment. Finally, the output phase describes the relationship(s) developed by the calibration. It includes generation of calibration curves or factors and/or derivation of confidence limits or precision and accuracy statements.

4.11.2.1 The Input Phase--

One of the most important decisions made in determining a calibration scheme is that choice of the reference material used in the calibration process. Standards include everything from permeation devices and pressurized cylinders to orchard leaves and bovine liver (Table 1). Most are already prepared, however, many are generated in situ.

Since the standard or reference material is the authority against which the calibration relationship (input vs. output) is developed, it must be of the highest available quality and be characterized to the maximum extent possible. In the United States, the National Bureau of Standards holds the position of final authority in the preparation of many reference materials. Their Standard Reference Material series contains the best standards of their type. Therefore, use of NBS-SRM's completely fulfills the requirements of high quality and full characterization necessary in a standard. However, since SRM's are handmade and individually characterized by lot, they are expensive and often in short supply. Therefore, it is generally desirable to employ secondary standards as the actual calibration standards. One SRM is thus maintained as a "calibration standard for the calibration standards."

ANALYTE	MATRIX	NOMINAL VALUE	
Analyzed Gases			
O_2	N_2	21	Mol%
CH_4	air	1, 10	ppm
CH_4	air	4	ppm
C_3H_8		1	ppm
SO_2	N_2	430,940,1500,2500	ppm
C_3H_8	air	3, 10, 50, 100, 500	ppm
CO_2	N_2	1, 7, 14	Mol%
CO	N_2	10, 50, 100, 500, 1000	ppm
NO	N_2	50, 100, 250, 500, 1000	ppm
CO	air	10, 20, 50	ppm
CO_2	N_2	.5, 1.5, 2, 2.5, 3, 3.5	Mol%
Analyzed Liquids			
S	Residual Fuel Oil	.3, 1, 2	wt.%
S	Distillate Fuel Oil	.2	wt.%
Trace Elements	Fuel Oil	---	---
Pb	Reference Fuel	12, 20, 28, 775	µg/g
Hg	Water	1.5 1.1	µg/mL µg/mL
Trace Elements	Water	---	---
Permeation Tubes			
SO_2	2, 5, 10 cm	.6, 1.5, 3	µg/min @ 25°C
NO_2		.5 - 1.5	µg/min @ 25°C

ANALYTE	MATRIX	NOMINAL VALUE	
Solid Materials			
Cr	Brewers Yeast	---	
K, Ca, P +	Spinach	---	
Pb, Hg +	Orchard Leaves	---	
K, Ca, P +	Tomato Leaves	---	
Ca, K, P +	Pine Needles	---	
Pb, Ag, +	Bovine Liver	---	
Pb	Lead-base Paint	12	wt.%
Hg	Coal	---	
S, Ash	Coal	---	
Hg, Pb +	Coal	---	
Pb, Hg +	Fly Ash	---	
COD, N, PO_4 +	River Sediment	---	
Industrial Hygiene Standards			
Benzene	on charcoal	---	
M-Xylene	on charcoal	---	
p-Dioxane	on charcoal	---	
1,2-Dichloro-ethane	on charcoal	---	
Chloroform	on charcoal	---	
Trichloro-ethylene	on charcoal	---	
Carbon Tetra-chloride	on charcoal	---	
F	Freeze-dried urine	.8, 7	µg/L
Hg	Freeze-dried urine	.05, 3	µg/L
Be	Filter Media	---	---
Pb, Cd, Zn, Mn	Filter Media	---	---
Quartz	Filter Media	---	---

TABLE 1. Currently available NBS-SRM's for environmental research and control

Whenever a secondary standard is employed in a calibration, it is necessary that a pathway (i.e., traceability) showing the relationship of the working standard to a standard of higher quality be established and maintained. Certain EPA regulations now specify traceability of calibration standards to NBS-SRM's [21,22] and it is likely that this requirement will appear in future regulations. It is, therefore, recommended that all HERL/RTP calibration procedures specify that calibration standards be traceable to NBS standards insofar as possible.

Some of the currently available standard reference materials provided by NBS are listed in Table 1. A listing of SRM's, complete with prices, is published in reference 19.

Unfortunately, NBS does not supply SRM's for every measurement process; in fact, there are no SRM's available at this time for many common measurement processes routinely used at HERL/RTP. In these cases, the investigator must use the "best available" calibration standard or, in some cases, devise a standard. These standards must also meet the requirements of high quality and careful characterization applicable to SRM's. High quality standards may be obtained by using raw materials of known high quality for construction or preparation. Careful characterization of such standards involves rigorous characterization to establish the "true value" of the reference material. Such testing includes repeated analysis of the standard, analysis by more than one analyst or technique, round-robin interlaboratory analyses, etc., to establish the true value within known limits of precision.

One further caution should be noted with respect to the use and handling of calibration standards. They must be used and handled under their specified conditions. It is a matter of record that many calibration data contain errors induced by incorrect handling of standards. The following list delineates some of the more common techniques ignored in the use of such standards:

a. Permeation devices must be used and stored under carefully specified environmental conditions of humidity [23], temperature [24], and protected from possible environmental contaminants [23].

b. Certain gases in pressurized cylinders require special procedures for regulator installation to prevent cylinder and regulator contamination with atmospheric oxygen or moisture (e.g., nitric oxide in nitrogen must not be contaminated with atmospheric oxygen) [25].

c. Electronic standards frequently require periods of several hours for stabilization of output (e.g., ozone generators).

d. Most solid standards (e.g., powdered chemicals such as potassium iodate or sodium sulfate and powdered mixtures such as orchard leaves or coal) require conditioning at a specified humidity prior to weighing.

These examples illustrate some potential mistreatment of otherwise valid calibration standards. The point to be emphasized is that users of standards should be intimately familiar with specified use conditions for each standard. It is imperative that this point be recognized if high quality data are to be obtained from the measurement process.

4.11.2.2 The Operation Phase--

During the operations phase of a calibration, the measurement process is calibrated or characterized against a standard. A written calibration procedure describing the individual steps by which the calibration is accomplished is required. Calibration procedures may be prepared in-house by qualified personnel, may be derived from instrument or process manufacturer's instructions, or may be found in sources such as ASTM Standards [26]. These procedures should be subjected to document control as outlined in Section 4.12 to assure that the latest revisions are being utilized.

Personnel actually performing a calibration should be qualified to do so. They should be intimately familiar with the measurement process as well as the calibration procedure. Their qualifications to calibrate should be demonstrated to a person of higher authority who is also qualified to perform the calibration and who has sign-off responsibility for the ability of the person to perform the calibration.

An aspect of the calibration operation that is often overlooked is the calibration of support equipment. The use of a high quailty calibration standard has already been discussed. However, most calibration procedures utilize equipment and/or reagents in addition to the standard(s). All such reagents and equipment should have been subjected to calibration prior to use in the procedure. The quality of the calibration is directly related to the quality of the data derived using any such equipment.

4.11.2.3 The Output Phase--

After the calibration of a measurement process, the derived relationship between the known input and the measurement process output must be depicted in a useable manner. The relationship may take the form of a calibration curve, a correction table, or a calibration factor or factors. Whatever method is chosen, the input/output relationship should be accurately expressed in a useable manner.

One of the most popular methods of expressing the calibration relationship is the calibration curve. In this method, the known values label the abscissa (x-axis) and the process outputs label the ordinate (y-axis). The calibration input/output pairs are plotted and an appropriate curve is used to connect the points. A minimum of five such points is necessary to adequately describe the curve that should cover the range of interest in the measurement process. A typical instrument calibration curve is shown in Figure 6.

Calibration curves are often linear; however, some take a nonlinear form. For this reason, the data should actually be plotted. If the curve is clearly linear, the technique of linear regression becomes useful. This technique is described in most statistics books and is valuable because it:

a. Allows more precise interpretation of curve data.

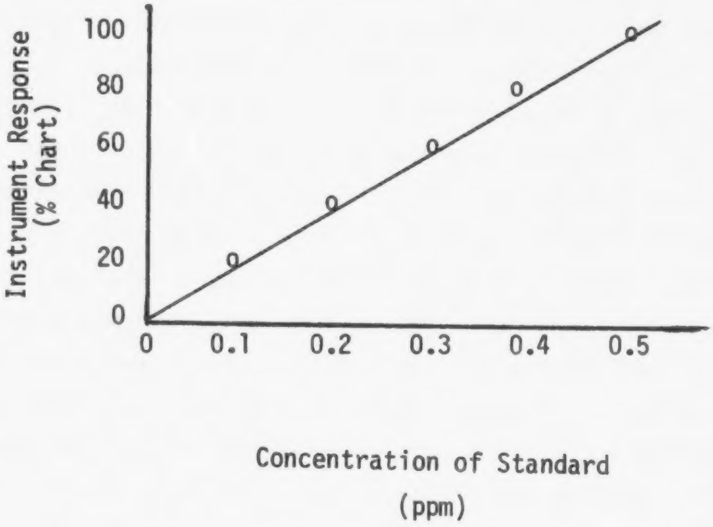

Figure 6. Typical calibration curve.

b. Allows all personnel to derive the same line from the data.

c. Provides a mathematical equation for calculating input or output data from the curve.

Regression analyses, including calculation of the standard error of the estimate, allow one to make statements concerning the precision of the calibration. However, nonlinear data will yield linear parameters when subjected to a linear regression analysis. Regression analysis should, therefore, include the calculation and evaluation of the correlation coefficient. Deviation of this coefficient from unity (1.0000) by any appreciable extent indicates that the data should be carefully reexamined to establish their linearity.

Experience has shown that almost all calibration curves contain nonlinear portions. Often, the nonlinear portions lie outside the range of interest and linear techniques are reliable as long as the working range is limited to those portions that are demonstrably linear.

With the advent of readily available microprocessors and programmable pocket calculators, it is becoming increasingly easier to perform regression analyses on nonlinear systems. This type of analysis provides the same advantages as linear techniques and should be seriously considered as a viable calibration data examination technique.

4.12 DOCUMENTATION CONTROL

Operating procedures for task measurement activities should be clearly documented and available to task operating personnel. A formal procedure for ensuring that procedural and system changes are incorporated into existing documentation and that those changes result in corresponding changes in the habits of operating personnel is essential.

Reference 3 clearly describes a comprehensive, practical document control indexing format appropriate for use within EPA laboratories.

It has the advantages that only current versions of documentation are generally retained, and updating may occur at any time. An example of the information placed in the upper right-hand corner of each page is as follows:

Section No.	2.12
Revision No.	0
Date	September 27, 1977
Page	1 of 5

(Note that the date given is the date of the revision.)

4.13 CONFIGURATION CONTROL

An adequate program of equipment/hardware configuration control (e.g., equipment location, environment, component alteration and/or replacement) will readily permit tracking all changes that are made to a data-producing system that may affect data quality. This applies to individual instruments as well as to entire data acquisition systems.

Authorization for configuration changes should be limited to one person, preferably the project officer, to assure that all changes to the facility (e.g., replacement of an electronics board in an analyzer) are properly documented and communicated. This documentation and communication is essential in understanding and explaining shifts in data patterns following such changes. It will also ensure that all involved personnel are notified of the changes, and that the proper procedures required by the changes (e.g., recalibration of analyzers) are initiated. Finally, it will provide a convenient way of assuring that all preventive maintenance procedures are performed on schedule.

For extensive systems, such variables as sampling site changes and monitoring instrument replacements should be recorded similarly to calibration and maintenance (Sections 4.10 and 4.11), i.e., in a bound, page-numbered notebook reserved for this purpose.

Configuration control for the laboratory environment is fully as important as for extensive monitoring systems. It includes instrument location in the laboratory as well as modifications (e.g., sample holder of different design) that affect measurement data. Temporary and/or permanent equipment configuration changes should be made only when the effect is well characterized and demonstrated to improve data quality.

4.14 DATA VALIDATION

Data validation may be defined as a systematic procedure whereby data are filtered and accepted or rejected based on a set of criteria for providing assurance of the validity (accuracy, precision, representativeness, completeness) of data prior to their ultimate use [3]. Criteria for each application of data validation techniques should be documented and implemented for all task data. Automated data acquisition systems are particularly suited for extensively comparing reported data values with earlier stored values of the same parameter and establishing and updating such statistics as parameter mean and standard deviation. Similarly, checks for data completeness, calibration performance, signal levels within reliable measurement range (i.e., above minimum detectable and below saturation levels), etc., should be designed into data validation systems.

Data validation must be defined with reference to the requirements of each task. Frequently, laboratory data validation relies on the highly trained professional judgment of the investigator or technician. However, to rely on such capabilities in a routine monitoring system situation invites disaster. In both situations, the data should be flagged but not discarded unless there is definitely identifiable error (e.g., an obvious and documented equipment malfunction).

In a laboratory environment, operating personnel who are alert and adequately trained regularly perform this type of screening as they manually collect data. This requires particular attention that valid data are not rejected without adequate reason. Data should not be rejected "because they don't look right" or other similarly subjective reasons; it is generally the case that such data are frequently valuable as the particular model is developed to a higher level of sophistication.

In either the laboratory environment or the complex data acquisition system, provision should be made for regular review of the appropriateness of the specific validation criteria. This analysis should include both technical and professional inputs in order to keep a proper balance of theoretical and practical considerations in the setting of limits on the data. In all cases, data validation procedures should not be permitted to delete raw data, but only to flag it when a clearly stated validation criterion is exceeded.

4.15 FEEDBACK AND CORRECTIVE ACTION

For each task, a system for deleting, reporting, and correcting problems that may be detrimental to data quality must be established. As noted in reference 3, this system "...can be casual when the organization is small or the problems few. When this is not the case ...action documentation and status records are required." The exact system design should accommodate the conflicting needs for (a) quick response and (b) thorough communication and documentation of the problem and its solution. Complex data acquisition systems require a formalized closed-loop system with standard forms for various stages of the problem and its solution. In a laboratory context, however, if a "fix" is not immediately apparent, direct contact between the project officer and the involved technician may be the most effective "system." An effective system will eliminate the causes of malfunctions before they occur. With this approach, corrective action becomes preventive, and the data from the process assume increasingly higher quality and greater reliability.

An important aspect in improving the potential for effective feedback and corrective action in task personnel is a quality assurance briefing (see Section 4.3). The purpose of this briefing is to make each individual involved in the task aware of how his personal contribution to the task affects its overall data quality. Such briefings certainly should take place during the opening phases of the task, and should probably be continued at specified intervals throughout the task life.

During the initial briefings, personnel could be exposed to the answers to such questions as:

-- What is the purpose of this task?
-- How do you fit into the overall task pattern?
-- How does your work affect task data quality?
-- What can you do to improve task data quality?

These briefings provide an excellent opportunity to establish and maintain an active employee-management feedback loop. Since the bench-level personnel are the best observers of routine task operations, they are also the most likely to detect disturbances. With an effective feedback loop in operation, management can quickly become aware of fluctuations that might otherwise go undetected. In general, it is important to impress on task personnel that any contribution to data quality is important. Their daily conduct literally controls task data quality.

Additional feedback systems should be established, at least informally. For example, the discovery of an impure substance by one investigator should be communicated to all other users of the particular substance as rapidly as possible. This can be facilitated by the use of adequate stockroom records.

A description of the problems, solution of the problems, and estimates of the effect of the problems on data quality should be made available to appropriate management on a regular basis.

4.16 DATA PROCESSING AND ANALYSIS

Data from health-effects research are rarely, if ever, used in the form in which they are recorded. The initial phase of data processing is to convert the data into a form suitable for conceptual manipulation and possibly perform preliminary statistical and other calculations. These intermediate results are then analyzed in terms of the particular model of interest to the investigator. Each of these transformations of the raw, observed data is made by a manually or electronically programmed series of manipulations. Hence, each transformation is a potential source of error in the final result. The automated analysis of large amounts of data thus carries the inherent potential for significant error, quite apart from experimental errors, due to the processing analysis functions.

Less obvious are the routine errors of transferring data from a notebook to machine-readable form (e.g., punched cards). Typical error rates for each transfer may exceed 5 percent. When several manual transfers are necessary, the quality of even the most carefully checked data degrades quickly and significantly.

Statistical analysis must be used judiciously. The validity of statistical techniques depends on their proper application to the particular experiment. In this context, regular contact with an in-house statistician who is intimately familiar with the study is essential.

The overall reliability of contemporary computer hardware systems is extremely high, due to various routine internal (to the machine) auditing checks. A major source of error may be traced to the software, which provides detailed instructions for operation of the hardware. Typical errors may generally be traced to insufficient testing of the program during the development stage or improper application by the user. Either condition is difficult to detect due to the wide range of values that may be supplied to a program for processing and that cause no hardware detectable error. The only insurance currently available against the "Garbage In, Garbage Out" problem is for each user to exercise his or her best professional capabilities to estimate reasonable results. If reasonable results are not produced by the software system, a concerted effort should be made to determine the exact source of the discrepancy.

The potential for such software problems is greater with increased use of locally (i.e., within laboratory group) written programs for individual minicomputers and microcomputers. In addition to verification of the proper handling of "good data," extensive testing of the proper handling of "bad data" (i.e., data containing some representative, anticipated errors) should be performed over the complete range of possible values and thoroughly documented. Consultation with the Data Management Staff in properly testing and debugging these programs will be cost-effective in terms of accurate and efficient data reduction.

4.17 REPORT DESIGN

The most visible product of a research task is the document(s) which comprises the report of the important findings. Publication guidelines applicable to the HERL research reports are available [27,28]; minimum technical contents for nonclinical laboratory reports and health effects research have been promulgated [9] and are shown in Figure 7.

As in all scientific research reports, and within the indicated consistent style stipulations [27,28], the report should be concise and complete, with adequate discussion of the important technical aspects of the research to permit a qualified professional to duplicate it. Adequate data should be included to permit at least partial calculation of important results. The conclusions, based on the data, and the reasoning to support those conclusions should be clearly stated. As much graphical and illustrative data correlation (with supporting tables, as appropriate) should be used as is feasible. Error estimates should be included with all quantitative and qualitative values reported, as well as the basis upon which the estimates were made.

Much of the research conducted under the auspices of HERL/RTP is highly specialized and frequently at the forefront of the technology, yet few of the individuals who make up the audience for the reports are specialists in the particular technical area. For this reason, the purpose(s) and conclusion(s) of the research should be stated as clearly as possible (see Section 4.2). The estimated errors, as well as the limits of applicability of results, should be stated in such a way as

(A) Name and address of the facility performing the study and the dates on which the study was initiated and completed.

(B) Objectives and procedures stated in the sponsor-approved protocol, including any changes in the original protocol including justification(s).

(C) Statistical methods employed for analyzing the data.

(D) The test and control substances identified by name, chemical abstract (CAS) number or code number, strength, purity, and composition or other appropriate characteristics.

(E) Stability of the test and control substances under the conditions of administration and storage.

(F) A description of the methods used.

(G) A description of the test system used. Where applicable, the final report must include the number of animals used, sex, body weight range, source of supply, species, strain and substrain, age, and procedure used for identification.

(H) A description of the dosage, dosage regimen, route of administration, and duration.

(I) A description of all circumstances that may have affected the quality or integrity of the data.

(J) The name of the study director, the names of the other scientists or professionals, and the names of all supervisory personnel, involved in the study.

(K) A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.

(L) The signed and dated reports of each of the individual scientists or other professionals involved in the study.

(M) The locations where all specimens, raw data, and the final report are to be stored.

(N) The statement prepared and signed by the quality assurance unit.

Figure 7. Minimum report technical content for EPA health effects tests [9].

to minimize misinterpretation. Application of the results to alternative theories (models) should be provided, with indication of the rationale used in reaching the stated conclusions rather than the alternative conclusions.

Quality control and quality assurance activities should be discussed in as much detail as possible. This discussion should permit the specialist and nonspecialist alike to correctly assess the level of the quality assurance effort invested in the research. This should, in addition, permit subjective evaluation of the validity and accuracy of the reported results and conclusions.

SECTION 5

DATA QUALITY ASSURANCE FOR RESEARCH PROJECTS

The technical discussion to this point has focused on quality control aspects of the quality assurance plan that influence test data quality from the perspective of operating personnel (or organization, in the case of extramural research). In this section, the discussion focuses on quality assurance aspects of the QA plans from the perspective of personnel other than operating personnel. The fundamental concept is that the project officer has at his disposal a variety of probes, or checks, on data quality quite independent of the functioning of the task research system. The choice of suitable probes, and their applications to the measurement system, is the project officer's, with the support of the HERL/RTP QA organization.

5.1 QUANTITATIVE ESTIMATES OF DATA QUALITY

Quantitative measurements and comparisons (i.e., quantitative audits) provide the best possible objective estimates of data quality--insofar as they are available. Current efforts by the National Bureau of Standards are resulting in the relatively rapid production of new environmentally related Standard Reference Materials (NBS-SRM's). A current catalog of NBS-SRM's [18,19] may be obtained from:

Office of Standard Reference Data
National Bureau of Standards
Washington, D.C. 20234

In addition, the World Health Organization maintains information on worldwide sources of biological standards [20].

Appropriate use of the available reference materials by the project officer can provide an objective measure of specific parameter data quality. A variety of techniques, all of which should be designed as blinds (i.e., with operating personnel unaware of the nature of the reference sample) are available. Direct analysis of the reference material and routine duplicate analysis of samples (one of which is "spiked" with a known amount of the reference material), are two possible uses of reference materials in analytical systems for the evaluation of solution concentration, aerosol characterization, etc.

Unfortunately, NBS-SRM's do not exist for many measurements of interest. In such cases, techniques should be devised for probing the quality of the task research system. Round-robin analysis of aliquots of a single sample may be performed by any number of laboratories. While accuracy (i.e., deviation from a "true" value) cannot be measured, an estimate of analytical variability (precision) is available. For labile samples, collaborative (side-by-side) analysis may be used (e.g., several technicians would count normal cells on a set of plates). This is equivalent to the round-robin test, but is performed at one location and at approximately the same time. To give a measure of various research system components' variability, interlaboratory and intralaboratory analysis/measurement programs may be designed. In this case, it is important that the statistical design of such testing recognize such aspects as operating shift changes, diurnal biological changes, and other nonrandom variability in the sample(s) and total measurement system.

5.2 QUALITATIVE ESTIMATES OF DATA QUALITY

In addition to the various quantitative probes available to a taskmaster, there are also qualitative probes of task research data quality. The comparison, rather than between two numerical values, is between the proposed and executed(ing) plans.

Thus the protocol (or work plan in the case of extramural support) is a statement of the reasoned plans of the operating organization. From qualitative measures of data quality (i.e., quantitative or system audit), an individual, independent of the operating organization or group, compares the planned activities with what is observed to occur.

While complete agreement is no guarantee of high quality data, discrepancies are an indication that all is not well, that the task is not under the control of the project officer as it should be. Thus, the qualitative audit includes consideration of the execution of the points addressed in the protocol (which should be essentially the points covered in Section 4): Are data actually being collected according to the statistical design; are operating personnel properly qualified for their responsibilities; are records properly recorded and maintained, etc.

In summary, the project officer has available various quantitative and qualitative probes to effectively demonstrate and document the quality of data being produced in a task.

Page

6.0 GUIDELINES FOR ATMOSPHERE GENERATION AND MONITORING...... 6-1
- 6.1 Introduction.. 6-1
- 6.2 Atmosphere Generation................................ 6-1
  - 6.2.1 General Considerations........................ 6-2
  - 6.2.2 Particulate or Aerosol Atmospheres........... 6-4
- 6.3 Sample Collection and Analysis....................... 6-6
  - 6.3.1 Introduction.................................. 6-6
  - 6.3.2 Sample Representativity....................... 6-7
  - 6.3.3 Physical Characterization of the Atmosphere.. 6-8
  - 6.3.4 Sample Quantity............................... 6-8
  - 6.3.5 Sample Handling and Storage.................. 6-9
  - 6.3.6 Recommendations for Sampling and Analysis of Selected Pollutants...................... 6-10
    - 6.3.6.1 Sulfur Dioxide (SO_2)................ 6-10
    - 6.3.6.2 Nitrogen Dioxide (NO_2).............. 6-13
    - 6.3.6.3 Photochemical Oxidants.............. 6-14
    - 6.3.6.4 Carbon Monoxide (CO)................ 6-14
    - 6.3.6.5 Hydrocarbons (HC)................... 6-15
    - 6.3.6.6 Peroxyacetyl Nitrate (PAN).......... 6-16
    - 6.3.6.7 Total Suspended Particulate Matter.. 6-16
    - 6.3.6.8 Sulfuric Acid Mist.................. 6-17
    - 6.3.6.9 Sulfates............................ 6-17

SECTION 6

GUIDELINES FOR ATMOSPHERE GENERATION AND MONITORING

6.1 INTRODUCTION

In the HERL/RTP exposure facilities and in population studies, the effects of various atmospheric pollutants on test subjects are evaluated. These pollutants are in gaseous and/or aerosol form. The exposure facilities are used to study the effects of synthetic atmospheres on humans and other test subjects. Population studies evaluate the effects of the natural atmosphere on humans.

The generation of synthetic atmospheres and the monitoring of both artificial and natural atmospheres are extremely complex tasks. Guidelines for quality assurance planning for air pollution measurement systems [3], and ambient air methods [4] have been developed by EPA. The goal of these tasks is to produce high quality exposure effects data, hence the details of generation, sampling, and analysis techniques must be considered within the quality assurance plans. In human exposure, quality assurance planning begins with subject safety, continues in the experimental tests, and finally provides the basis for estimating the confidence limits on the exposure-effect relationships.

6.2 ATMOSPHERE GENERATION

The test atmosphere in an exposure chamber must be well characterized, in terms of both the composition and the concentration of the components. The exposure experiments may run from a few hours duration to several consecutive days. The total dose, as well as the instantaneous concentration level, is important in such experiments. Therefore, it is essential that the exposure source output be stable over the exposure period. Since synergistic effects can complicate interpretation of the experiments, care must be taken to assure that the desired species are present and that interferents are controlled and/or monitored. The changes in the composition due to loss of specific species or generation of another species by physical or chemical reaction must be taken into account.

6.2.1 General Considerations

Specific test pollutants (gaseous and/or particulate) are produced by a source mixed with diluent gas and then introduced into the space surrounding the test subject. Gaseous pollutants are usually obtained from high pressure gas cylinders, although some are produced in situ (e.g., ozone from ultraviolet irradiation). Occasionally, pollutants in ambient concentrations are obtained from permeation tubes (e.g., SO_2, NO_2, H_2S, CH_3SH). Aerosols may be generated by nebulizing a solution (or a suspension) of known composition. Aerosols in solid form may be obtained from a "dust-feeder" type of apparatus such as the Wright Dust-Feeder [29,30].

To assure the composition of the atmosphere, the source and the background atmosphere into which the pollutant is released must be well-characterized and stable. Reactivity of the pollutant with the test chamber (including the delivery system) must be characterized and documented.

Since atmospheres are generally prepared by introduction of a specific amount of pollutant into a known volume of background air, the quality of this background air is vital. Particulate matter, organic vapors, and other gases should be removed by appropriate filters, adsorbents, etc., prior to pollutant introduction. A schedule for the periodic replacement of these filters and absorbent elements should be established as indicated in Sections 4.4 and 4.10.

One of the frequently neglected "other gases" is water vapor. The humidity of the test atmosphere is an important variable, especially when atmospheres containing particulate matter are being generated. Surface reactions on particulates and aerosol composition are strongly dependent on the amount of water vapor present. All moisture should be removed from the background air using a mechanical dryer and absorbent. The air can then be rehumidified to a specified level by the addition of steam or a water spray. Test subject humidity requirements (e.g., rodents, ca. 50 percent) must be considered in determining the relative humidity finally obtained.

Since the test atmosphere is prepared by mixing pollutants and air in proportions described by the ratios of their volumetric flow rates, the accurate measurement of each of these flows becomes critical. Flow measuring devices should be properly calibrated and operated. The pressure and temperature of gases at the flow measuring devices must be stable and known. It must be realized that small absolute errors in the measurement of the characteristically low pollutant flow rates result in large relative errors in pollutant concentration. In addition, after pollutant and air flows have been combined, it is important to provide for good mixing of the two components in order to assure a homogeneous atmosphere.

Finally, it is important to characterize the test atmosphere as it encounters the test subject, i.e., spatial as well as temporal characterization. This characterization provides data concerning the actual exposure conditions. It is also helpful to characterize the atmosphere as it leaves the source since this information is useful in the early detection of harmful levels of pollutants resulting from source malfunctions. Early detection allows diversion of the defective atmosphere before it reaches the test subject. Interaction between individual components of test atmospheres, always a potential source of error in atmosphere generation, can be minimized by careful attention to parameters such as composition, concentration, and residence time. Interaction between the atmosphere and conduit or chamber walls can also be a source of error. This is especially true for aerosols and reactive gases such as ozone and sulfur dioxide. Even the test subject may interact with the atmosphere in an unexpected and undesirable manner (e.g., NH_3 from animal excreta). For valid data to be accumulated from an experiment, each of the interactions that may occur must be carefully examined and controlled by the project officer.

6.2.2 Particulate or Aerosol Atmospheres

Atmospheres that contain generated particulate matter or aerosols exhibit so many specialized problems that they warrant separate discussion. Since the dose of inhaled particles is mass- and size-dependent, knowledge of both the mass of the particles and their size distribution are needed in order to characterize the dose level. If the aerosol atmosphere is a mixture of several particulate components, the size distribution of each should be characterized.

As was mentioned earlier, aerosols may be obtained by nebulization of a solution (or a suspension) of known composition. Deviations in the aerosol characteristics may result from inadequate flow control of the nebulized air, excess loss of solvent, and cooling of the solution due to solvent evaporation. Circulation of the solution from an external large reservoir may be used to avoid the problems due to solvent evaporation. In the Wright Dust-Feeder, lack of homogeneity in the powder plug may produce deviation in aerosol output [31].

Characteristics of an aerosol may change due to particle-gas or particle-particle interactions. The particle-gas interactions in hygroscopic aerosols result in evaporation or growth of particles (for examples see reference 32). In salt aerosols, humidities above 75 percent will generally result in growth of the particles. This growth process is extremely rapid and can lead to a several-fold particle size increase at high humidities (>90 percent). In acid aerosols humidities below 20 percent can produce change in the other direction due to evaporation. As a general rule, and dependent on test subject health parameters, humidity should be maintained constant between 20 percent and 70 percent to avoid particle growth or evaporation losses.

Particle-particle interactions resulting in coagulation are dependent upon the particle size and concentration. Coagulation can lead to significant errors for dense aerosols. In general, if the concentration is less than 10^5 particles/cm^3, coagulation may be neglected. Other factors leading to coagulation are turbulent mixing and extreme polydispersity. Charges on particles also significantly influence aerosol behavior. An aerosol generated by nebulization may require charge neutralization. This will avoid the uncertainty of the effect of charge on particle-particle interaction and deposition on surfaces.

Methods for characterization of aerosol size and concentration are based on a variety of principles. Interconversion between two methods is not usually possible without introduction of significant errors. If an aerosol is used for an inhalation study, the aerodynamic size distribution based on mass is appropriate. To obtain this information, inertial classification of particles by a method such as impaction is necessary. However, in various size ranges, other methods based on electrical mobility, microscopic, or light scattering analysis may be needed to characterize the aerosol. Conversion of data from these methods into aerodynamic size should follow recognized procedures such as those described in reference 33. An estimate of the errors involved should accompany the conversion.

After the aerosol has been generated and characterized at the source, it is delivered to the test subject. Certain precautions and pretests should be taken to prevent significant change in the atmosphere before it reaches the subject.

Losses of the aerosol component en route to the exposure chamber can be significant. The most common cause of particle loss is deposition on conduit walls. This deposition of particles on surfaces is due to sedimentation, inertia, and diffusion processes: the extent and nature of particle loss is size-dependent. In polydisperse aerosols the deposition loss of particulates will affect the particle size distribution as well as the concentration. In general, large particles over a few microns are preferentially lost by sedimentation and inertia. These effects can be minimized by using high flow velocities and by avoiding bends or sudden transitions. Because of this tendency toward deposition, it is extremely important that aerosol atmospheres be finally characterized immediately before they encounter the test subject.

If the atmosphere contains particles larger than 1 μm in diameter, lack of homogeneity in the chamber may be significant.

Segregation may occur due to sedimentation or bypassing the inlet and outlet. Distribution of the incoming test atmosphere over as broad an area as possible would minimize the flow channeling problems. Sedimentation effects may be minimized by a vertically downward movement of the test atmosphere. Even with these precautions, segregation may occur. Again, this tendency necessitates characterization of the aerosol at the test subject. The sampling position for this characterization must represent the same location and elevation in the chamber as the test subject. This will assure the characterization of that portion of the atmosphere that actually reaches the subject.

6.3 SAMPLE COLLECTION AND ANALYSIS

6.3.1 Introduction

Collection of a representative sample is of utmost importance in any measurement process as noted in Section 4.7. The analytical results may be of excellent quality; however, if the sample is contaminated, degraded, or is otherwise not representative of the area or population under study, the relationship between the measured pollutant concentration and the response of exposed subjects will not be valid.

It is important to recognize that obtaining a representative sample is difficult, especially when components of the ambient air are measured. For this reason the processes of sample collection and analysis should be included in the experimental design (see Section 4.2). Sampling methodology and the number of samples required should be established prior to beginning the task.

Ambient air studies frequently deal with large populations and extended airshed areas, which cannot be thoroughly monitored. Thus, statistical sampling techniques are generally required. The number and size of "blanks," control groups, and samples taken from the background should be carefully evaluated. Calibration, instrument spanning, and audits also have an impact on sample collection and analysis efforts.

In most studies, more than one pollutant or parameter will be measured. During the experimental design phase, the requirement for measurement of co-occurring pollutants should be addressed. Important parameters such as humidity, temperature, and atmospheric pressure are also commonly measured.

6.3.2 Sample Representativity

A representative air sample of ambient air to which plants and animals may be exposed is difficult to obtain. Spatial and temporal aspects of sampling should be considered carefully prior to locating the sampling stations. A thorough background study in support of an ambient air monitoring program should include a study of source inventories, historical meteorology of the area, local topography, and examination of data from any preexisting air monitoring stations.

The point in space from which a sample is taken is an important variable. The sample should be collected in a location which is clearly representative of the air space being characterized. For example, if the objective of the study is to assess the effects of air quality on children, the sampling point might be located in a schoolyard or playground 1 to 1.5 meters above the ground. The inlet to the sampling probe must be located in such a way as to protect it from possible damage -- by the elements or by vandals -- yet out of the microenvironment of the sampling equipment. The use of mobile sampling equipment is often very helpful in locating proper sampling points and in surveying a large area at minimal expense.

The time frame in which a sample is taken also has a bearing on sample representativity. Generally, the longer the period of sampling, the better the sample will characterize the environment. However, the final decisions concerning sampling duration and frequency must be made with respect to the objectives of the task (see Section 4.2). If continuous or semicontinuous analyzers are used, concentration trends and any unusually high or low values will appear when the overall data are examined. On the other hand, if "grab" samples are collected for short periods of time, it is probable that very high or very low concentrations will be obtained which do not represent the subject's average exposure. If the experiment is well controlled (such as a captured air mass in a chamber), periodic (or grab) sampling can be utilized. However, even here with certain pollutants the use of grab samples is discouraged due to potential chemical degradation of the sample during transport and storage [34].

Sample integration is a helpful technique when one must collect an air sample for later analysis. In this process the sampling vessel is slowly filled with an air sample over a period of time. Again, sample integration must consider the stability of the sample with time, as well as the averaging of concentration fluctuations.

6.3.3 Physical Characterization of the Atmosphere

To obtain an accurate intercomparison of samples taken in various cities or air regimes, it is necessary to know some of the physical characteristics of the ambient or enclosed air mass. Such characteristics include temperature, barometric pressure, relative humidity, and perhaps wind speed, wind direction, and solar radiation. Additionally, knowledge of the temperature, pressure, and humidity within the analytical laboratory is necessary for correction of gas flow rates to standard temperature (25° C) and pressure (1 atmosphere). This is particularly important during the calibration and operation of analyzers and impinger systems.

6.3.4 Sample Quantity

A sufficient volume of air must be collected or passed to an instrument to obtain valid data. In the case of continuous analyzers, an excess volume of sample generally flows through a glass sampling manifold and the instrument's sampling line is attached to this manifold. An initial flow rate at least 50 percent in excess of that required by the analyzer(s) is recommended. If the sample flow is less than that demanded by an analyzer, the analyzer or sampling device will pull in room air and the sample will be diluted. Sufficient sample quantity is also needed during calibration. The rate of sample flow to a continuous analyzer should be identical to the flow established during calibration. That is, if an analyzer samples 200 cm^3/min during calibration, it should sample 200 cm^3/min during analysis of ambient air. The same is true of impinger samples in which air is bubbled through a chemical solution. In the case of impingers containing chemical solutions, a sufficient volume of sample must bubble through the solution to achieve a reliably detectable spectrophotometric or other response. For particulate collection devices (high volume samplers, cascade impactors, etc.) the flow must be that specified to achieve the entrainment of the desired particle sizes. The particle sampler must sample a sufficient length of time to build up sufficient deposit for accurate weighing and/or chemical analysis.

6.3.5 Sample Handling and Storage

Since many of the pollutants in ambient air are highly reactive, unstable species, they cannot be reliably collected and stored for later analysis. Ozone, oxides of nitrogen, peroxyacetyl nitrate (PAN), sulfur dioxide, and other sulfur species should be delivered directly from the ambient air to the analyzer or impinger through Teflon or glass tubing.

Other less reactive pollutants, such as carbon monoxide and hydrocarbons, may be stored for periods of several days prior to analysis. Teflon or Tedlar bags are adequate for carbon monoxide samples. Stainless steel or glass sampling containers are better for hydrocarbons. There may be no clear consensus in the scientific community as to the reactivity of a specific pollutant. In such cases, it is essential that it be determined and documented as part of the study if the conclusions are to be valid and defensible.

Particulate samples collected on glass fiber or other types of filters are often weighed and analyzed at a later date. For reproducible weight determinations, the filters must be conditioned at a constant relative humidity for a specified period prior to weighing for tare and gross weights.

In all cases, stored samples should be protected from unusually high temperatures and light. Some samples are best stored under refrigeration in the dark.

6.3.6 Recommendations for Sampling and Analysis of Selected Pollutants

Recommendations for the sampling and analysis of selected pollutants commonly found in ambient atmospheres follow. Included are suggestions and a summary (Table 2) for the six EPA criteria pollutants as well as other species of current interest to HERL/RTP personnel. This is not an exhaustive list--either of pollutants or of sampling and analysis methods. Rather, it is a list of some pollutants of current interest and their most often accepted analysis methods, major interferences, and calibration concepts (see reference 35 for additional pollutants).

6.3.6.1 Sulfur Dioxide (SO_2)--

The EPA reference method for determination of ambient levels of sulfur dioxide is the pararosanaline method. This manual, wet chemical method is a complex sampling and analysis procedure. EPA has accepted an automated version of this method that reduces the complexity of the analysis [36]. However, it is the continuous, instrumental methods currently available that produce the most data for the least professional time invested. These methods include coulometric, flame photometric, pulsed fluorescent, and second derivative spectroscopic detection of sulfur dioxide. The coulometric, pulsed fluorescent, and second derivative spectroscopic methods are specific for SO_2. The flame photometric method detects sulfur-containing species (e.g., SO_2, H_2S, R-SH); it can be made specific for SO_2 by inserting a scrubber cartridge into the sample inlet line.

POLLUTANT	MEASUREMENT METHOD	INTERFERRENTS	CALIBRATION	GENERATION
Ammonia (NH_3)	Indophenol Method	Certain Particulates	Standard Solution of NH_4^+	Cylinder and Dilution System
		Prefilters		
	Nitrite Method	Particulates containing ammonium salts		
Carbon Monoxide (CO)	NDIR (40 CFR, App. C)	H_2O, CO_2 in high conc. and other IR absorbers, near 2165 cm^{-1}	CO in N_2 (cylinders)	Cylinder and Dilution System
	Methanation/GC-FID	Non-methane hydrocarbons, depending on scrubber efficiency		
Hydrocarbons (THC)	GC-FID (40CFR, App. E)	Non-linear FID response to increasing carbon number	Methane cylinders	
	GC-MS	None	Standard cylinders of pure gases, and mixtures	
Hydrogen Sulfide (H_2S)	Methylene Blue	Strong oxidizing or reducing agents (e.g., SO_2)	Permeation system	Cylinder gas dilution with clean air
Nitrogen Dioxide (NO_2)	Chemiluminescence of NO with O_3 (40 CFR, App F)	CO_2, H_2O (33) PAN (34)	Cylinder NO in N_2 and gas phase titration to NO_2 (23)	Catalytic oxidation of NO (from cylinder)
Photochemical Oxidants (O_3)	colorimetric (NBKI)	SO_2, H_2S, NO_2, NO	O_3 (uv) generator	O_3 (uv) generator
	uv photometry		O_3 (uv) generator	O_3 (uv) generator
	chemiluminescence		O_3 (uv) generator	O_3 (uv) generator
	O_3-ethylene (40 CFR, App. D)	High humidity, at low O_3 concentrations	O_3 (uv) generator	O_3 (uv) generator
	O_3-rhodamine-B		O_3 (uv) generator	O_3 (uv) generator

Table 2. Summary of measurement methods for selected pollutants.

POLLUTANT	MEASUREMENT METHOD	INTERFERRENTS	CALIBRATION	GENERATION
Peroxyacetyl Nitrate (PAN)	GC-EC	Varying sample/standard moisture content	Also, photoanalysis of ethyl nitrite in oxygen (38)	Dilution of generated standard
Particulates (TSP)	High Volume Sampler (40 CFR, App. B)	Secondary particle formation		
		Gas-filter reactions		
Sulfates $(SO_4^=)$	Barium Methylthymol Blue	Anions complexing barium	Standard solution of $SO_4^=$	
Sulfur Dioxide (SO_2)	Pararosaniline (40 CFR, App. A)	None	SO_2 permeation system	Mixing of high-concentration SO_2 with clean air.
	Pulsed Fluorescence	Aromatic Hydrocarbons	SO_2 permeation system	Mixing of high-concentration SO_2 with clean air.
	Flame Photometric	Any sulfur-containing species	SO_2 permeation system	Mixing of high-concentration SO_2 with clean air.
		CO_2 (32)	SO_2 permeation system	Mixing of high-concentration SO_2 with clean air.
	Coulometric	Oxidizing (e.g., O_3, NO_2) species	SO_2 permeation system	Mixing of high-concentration SO_2 with clean air.
		Reducing (e.g., H_2S) species	SO_2 permeation system	Mixing of high-concentration SO_2 with clean air.
		Olefins	SO_2 permeation system	Mixing of high-concentration SO_2 with clean air.
Sulfuric Acid (H_2SO_4) Mist	Collection on filter	SO_2		

Table 2. Summary of measurement methods for selected pollutants (continued).

Some nonsulfur compounds do interfere with these methods. It is reported that differences between carbon dioxide concentration in the calibration/zero matrix and the sample matrix interfere with certain flame photometric detectors [37]. Hence, the CO_2 concentration in the calibration gas for this instrument should be matched to the CO_2 concentration expected in the sample. The pulsed fluorescence method will respond to certain aromatic hydrocarbons unless a special scrubber (referred to as a hydrocarbon cutter) is placed in the sample inlet line.

Calibration of these instruments is usually accomplished against a SO_2 permeation device. Although gas cylinders of SO_2 are widely used for calibrations of source level monitors, they are generally not employed for ambient level instruments due to stability problems.

6.3.6.2 Nitrogen Dioxide (NO_2)--

The EPA measurement principle for the determination of ambient nitrogen dioxide (NO_2) is based on the chemiluminescence produced by the oxidation of NO with ozone. The method is instrumental and continuous. These analyzers detect NO and total oxides of nitrogen (NO_x) directly. A readout of NO_2 concentration is provided indirectly by electronic subtraction. One automated and two manual wet chemical methods have recently been accepted by EPA as equivalent to the reference principle [24]. Neither the Christie (arsenite) method nor the TGS-ANSA method is affected by the interferences listed above, but they suffer from the difficulties inherent in all manual sampling and analysis methods.

Recently published research has indicated that this method is subject to interference from third-body quenching reactions including those with carbon dioxide and water vapor [38]. Research has also shown that the thermal converter (used in this method to reduce NO_2 to NO) can reduce nitrogen-containing compounds to NO. PAN is also converted with relatively high efficiency [39]. The efficiency of this converter should be determined frequently, especially when high concentrations of nitrogen dioxide are being analyzed.

Calibration of the NO and NO_x channels of the instrumental method is generally accomplished using bottled standards of nitric oxide in nitrogen. The NO_2 channel is calibrated by oxidizing some of the NO calibration standard to NO_2 before the gas is introduced into the instrument. This oxidation is accomplished by ozone gas phase titration (GPT). Calibration of this channel may also be accomplished against a NO_2 permeation device. Much helpful information on the calibration and use of chemiluminescence NO-NO_2-NO_x analyzers is available in an EPA technical assistance document [25].

6.3.6.3 Photochemical Oxidants--

Ozone (O_3) is the most often measured photochemical oxidant. Wet chemical methods can be employed for measurement of ozone. However, the recommended analytical procedures are the instrumental methods based on ultraviolet photometry, chemiluminescence from the reaction between ozone and ethylene, or chemiluminescence from the reaction between ozone and rhodamine-B.

Calibration is accomplished using an ozone generator. Its output is determined by gas phase titration of nitric oxide, ultraviolet photometry, neutral buffered potassium iodide, or boric acid buffered potassium iodide colorimetry.

6.3.6.4 Carbon Monoxide (CO)--

The EPA measurement principle for continuous monitoring of carbon monoxide in the atmosphere is nondispersive infrared spectrometry (NDIR). The principle is based on the absorption of infrared radiation by carbon monoxide in a nondispersive spectrophotometer. Another method is based on catalytic conversion of carbon monoxide to methane by hydrogenation. The methane is then sensed by a flame ionization detector.

The infrared adsorption spectrum of water is sufficiently similar to that of CO to interfere for NDIR measurements. In source level concentrations (e.g., 2000 ppm), CO_2 is also an interferent.

Calibration of such analyzers is by injection of carbon monoxide from standard cylinders. Steel cylinders have a tendency to react slowly with carbon monoxide, forming iron carbonyl. Because of this tendency, standards should be verified every 4 to 6 months by comparison to an NBS-traceable standard.

6.3.6.5 Hydrocarbons (HC)--

The EPA measurement principle for determination of hydrocarbons corrected for methane is an instrumental method based on gas chromatography with flame ionization detection. The method is designed to measure both total hydrocarbons and methane so that methane can be manually subtracted from the hydrocarbon analysis. No reference instruments are currently designated because of problems resulting from an inefficient methanator and a nonlinear detector. The instrument is usually calibrated on the basis of methane supplied from low-level standard reference tanks.

If analysis for specific hydrocarbons is sought, the chromatographic column-flame ionization detector approach is preferred. The specific compound is distinguished from others by introducing a known concentration of this hydrocarbon and determining its column retention time. The signal strength from the detector is correlated with concentration by introducing varying known concentrations of the hydrocarbon of interest. Permeation tubes containing certain hydrocarbons may be used to generate standards. Mixtures of hydrocarbons in air or other gases may also be purchased in cylinders.

The possibility of peaks from one or more compounds overlapping during chromatographic analysis increases with the complexity of the molecules. The extent of this problem should be investigated using several conditions and column packings.

Hydrocarbon samples may be collected in Teflon or Tedlar bags for later analysis. However, glass or stainless steel containers are preferred. Certain hydrocarbons and other organic compounds may be adsorbed on columns of polymeric material such as TENAX-GC and volatilized onto a chromatographic column at a later time.

For ultimate certainty in identification of hydrocarbons (and other organic species), the method of choice is the combination of gas chromatography and mass spectrometry.

6.3.6.6 Peroxyacetyl Nitrate (PAN)--

PAN is a photochemical oxidant often found in smoggy atmospheres. Measurement is generally by a gas chromatographic procedure employing an electron capture detector [40]. This method may be subject to interference from low sample moisture content unless the relative humidities of the samples and standards are controlled [41,42].

Standards may be synthesized by the photolysis of ethyl nitrite in oxygen [43]. The synthesized standard, however, is not a primary one and must be verified (e.g., by infrared spectroscopy).

6.3.6.7 Total Suspended Particulate Matter--

The EPA reference method for total suspended particulate (TSP) is the high volume sampler method. Air is drawn into a covered housing and through a filter by means of a high flow rate flower (1.0-1.7 m^3/min). This flow rate allows suspended particulates having diameters of less than 100 μm to pass to the filter surface. Accurate control of the flow rate is critical to obtaining a valid sample. The collection period for ambient air is generally 24 hours. The filter is conditioned to a fixed relative humidity and weighed before and after sampling. The net weight and total volume sampled are used to estimate average suspended particulate matter in terms of micrograms per cubic meter.

For experiments where smaller volumes of air are available for sampling, low flow rate filters and impactors may be useful. Because the emphasis here will probably be on chemical analysis and not weight, care must be exercised in selecting the filter media or impaction surface. The possibility of interferences in the analysis should be examined through background and blank analyses. Optical particle counters are available for continuously monitoring the number of particles and, in certain models, the size of particles. Manufacturers of such counters and size discriminators should be able to show how calibration was achieved.

6.3.6.8 Sulfuric Acid Mist--

Sulfuric acid mist may be collected on glass fiber or Teflon membrane filters if it is at low concentration levels (<50 mg/m^3) and no sulfur dioxide is present. The sample can then be extracted with deionized water and analyzed. When sulfur dioxide is present, it may be oxidized to sulfate by moisture or an oxidant on the filter surface thus interfering with the acid mist analysis.

6.3.6.9 Sulfates--

Airborne sulfates may be measured by analysis of the particulate matter taken from high volume filters. The technique is generally as described for the measurement of total suspended particulate matter (Section 6.3.6.7). Analysis of the collected particulate for sulfate is then performed using one of several available analytical techniques (e.g., turbidimetry, ion chromatography). To avoid unwanted formation of sulfates on the filter by reaction of SO_2, the pH of the filter must be controlled during manufacture to around pH 5. The analysis method usually recommended is the automated, wet chemical method based on the detection of the barium-methyl-thymol blue chelate [44].

Page

7.0 ANIMAL DOSING.. 7-1
    7.1 Preparation.. 7-1
        7.1.1 Preparation of Animals........................ 7-1
        7.1.2 Preparation of the Test Substance............. 7-2
        7.1.3 Preparation of the Control Substance.......... 7-3
        7.1.4 Preparation of the Vehicle.................... 7-4
        7.1.5 Mixing.. 7-4
    7.2 Administration..................................... 7-5

SECTION 7

ANIMAL DOSING

In addition to inhalation dosing described in the previous section, research within HERL/RTP requires the administration of a wide variety of solid and liquid test substances to animal subjects, primarily mammals. The dose-response data are the basis for subsequent analysis and evaluation; quality assurance must be included in the planning of dosing and activities as well as in the response analysis in order to assure the specified data quality. This section is intended to outline major considerations in animal dosing from which the project officer can develop task-specific plans for assuring the quality of animal dosing data.

7.1 PREPARATION

Planning for animal dosing should be aimed at adequately controlling and/or documenting dose parameters of the research, as well as the other parameters. A complicating factor is that animals respond emotionally (and thus biochemically) to a wide variety of stimuli in their total environment. This response is directly linked with subtle and complex biochemical changes, which may obscure or alter the parameters under study. Hence, the planning for animal dosing should attempt to address and control all aspects of the known environment which may effect these biochemical changes.

7.1.1 Preparation of Animals

Beyond the recommended receiving quarantine period, animals should be acclimated to the total test regimen insofar as possible. They should be exposed to routine environmental factors such as room temperature, lighting levels, and feeding schedules for a time sufficient to stabilize their responses. They should primarily be exposed to the animal handlers and appropriate handling routines, such as transportation from the animal care facility to the test laboratories. In this way, behavioral and biochemical changes may be reliably attributed to the testing program rather than changes in the environment.

7.1.2 Preparation of the Test Substance

As a primary mission, research at HERL/RTP involves test substances that are suspected of being highly toxic, carcinogenic, or otherwise injurious to humans. Hence, a primary consideration in planning must be the safety and protection of operating personnel from these test substances.

The screening of test substances and other supplies for purity and quality is treated in a general way in Section 4.6. Characterization of the test substances is even more critical, since the desired datum results from the interaction of the test substance with the various animals' complex biological systems. Uncharacterized changes, such as electrolyte composition or microbiological activity, will induce a pronounced response that will be misinterpreted as being caused by the test substance. The effects in terms of lost time, money, and credibility are obvious.

The test substance should be obtained in sufficient quantity to more than meet all requirements of the specific study and should be all from the same lot. In this way, adequate characterization will have extensive applicability and thus be economical of both time and money.

Characterization of the test substance in terms of purity is essential if the calculations for the dose--as delivered--are to have any validity. This may be performed in a number of ways; e.g., measuring an instrumental response of a standard preparation or measuring its effect on standard cell cultures. Two or more methods whose principles are unrelated should be used, as a minimum, to avoid the possibility of compensating effects. The exact methods of verification are determined by the research methods involved and by the professional expertise of the principal investigator.

Characterization of test substance impurities and "inactive substances" is a more difficult task, yet it is crucial to the correct interpretation of test results. Most suppliers make an effort to control the quality of their products, yet they frequently release an inferior product; neither are they in a position to precisely accommodate the research test requirements. For example, substitution of the potassium salt for the sodium salt of a substance is of minor concern to many chemists, yet there will be a significantly different response if it is injected intravenously into an animal. As part of appropriate experimental design, analysis for "inactive" components of the test substance must be planned. Provision should also be made for subsequent testing if incoming test results do not follow anticipated trends. While it is relatively straightforward to analyze for a specific substance, it is impossible to determine that all interferents are absent: only the expertise of the project officer and the informed observations of operating personnel are useable in minimizing, but not eliminating, this problem.

Additionally, an estimate of the homogeneity of the test substance should be made; the results of replicate analyses during acceptance testing and purity checks provide data from which the project officer can make this estimate. Subsequently, regular analysis during the life of a task will provide data from which an estimate of the stability of the test substance can be made.

By including provision for thorough characterization of the test substance, the research conclusions will be strengthened and doubts of their validity minimized.

7.1.3 Preparation of the Control Substances

Health research routinely involves groups, whether cell cultures or human subjects are involved. The concept of a control group involves comparison between two groups that are strictly equivalent, excepting only the active test substance. In order to compensate for deletion of the test substance, a control substance having similar properties must be used.

Since differenct substances cannot be strictly equivalent, the crux of the project officer's choice of the control substance is to decide what characteristics of the test sbustance are to be considered equivalent. This depends on the exact nature of the test, and requires case-by-case application of the project officer's expertise in conjunction with peers with whom he advises.

Specific considerations for the choice of control substance hinge on the precise nature of the test substance as well as the particular biological system under investigation. The cation may be significant to some systems but not to others. Microbiological contamination, on the other hand, will almost always be a consideration.

7.1.4 Preparation of the Vehicle

The choice and preparation of a vehicle depends on the solubility properties of particular test substances (and control substances) as well as intended route of administration. The possible pH range will differ for oral and intravenous administration, among other considerations. This aspect of dosing is generally a routine consideration, yet it is an important parameter in the overall dosing scheme, which should have the project officer's close attention especially during the planning stages of the task.

7.1.5 Mixing

As noted in Section 7.1.2, the majority of test substances encountered within HERL/RTP should be considered toxic, carcinogenic, or otherwise injurious to humans. Hence, plans for mixing must include explicit safeguards for the operating personnel and for test subjects.

Depending, again, on the intended route of administration, the mixing of the test/control substance with the vehicle to produce the dosing matrix follows varying procedures. For inspection, demonstrably homogeneous solutions are frequently used. Emulsions are also common, but they are less stable in terms of long-term homogeneity, and should be prepared as near to the time of use as possible [45]. Oral dosing, especially via feed, presents a complex set of problems in that the test substance is frequently mixed with dry feed, which only lightly and nonuniformly coats the pellets. The individual laboratory animal feeding schedule and total consumption are not as controllable as, for example, an injection. At the minimum, feed mixing should be consistently performed by one person according to a well-designed plan, and data should be collected regularly to characterize the uniformity and repeatability of this operation.

An analysis plan should be devised that suitably characterizes the total mixed system on a regular basis over the life of the task. This includes demonstration of the (lack of) interaction of the test/control substance and the vehicle. It also includes accumulation of data of this type at regular intervals throughout the course of the project.

In addition, in choosing the test substance, the control substance, and the vehicle, care must be taken that no inadvertent synergism is involved in inducing the response. During the experimental design phase, use of combinations of these components should be planned to demonstrate that synergism is not a factor.

7.2 Administration

The route of administration directly affects the dosing preparations. The available routes [45,46] are oral, intravenous, intraperitoneal, and subcutaneous. Each has its advantages and disadvantages, which must be evaluated in the context of the specific research study. Technical limitations to oral dosing include the complex interactions of the animal's digestive system with the dosing matrix. Similarly, intravenous injection implicitly involves the action of the liver system on the dosing matrix. Again, the choice of administration route is the decision of the project officer, who, in consulting with his peers, should arrive at a scientifically appropriate and defensible conclusion.

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